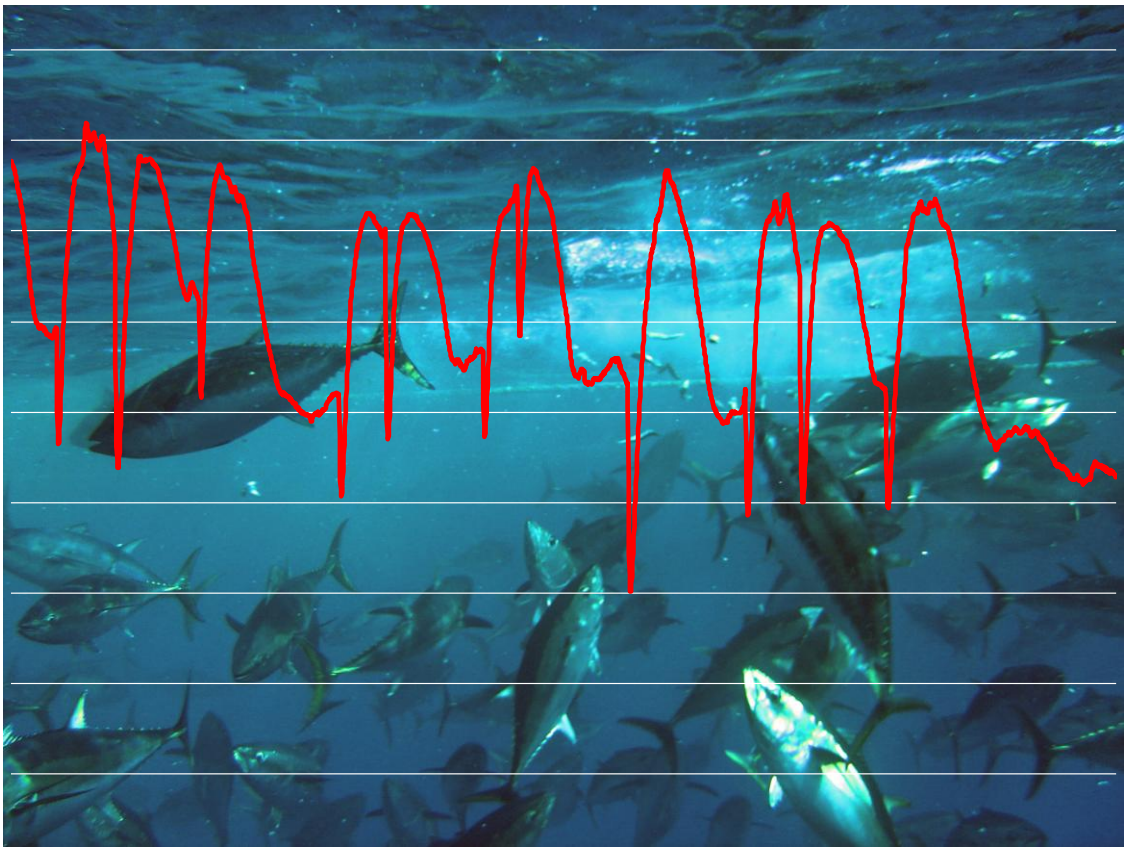
The measurement of visceral temperature patterns and implications for feeding practices in ranched southern bluefin tuna *Thunnus maccoyii*



David Ellis *DipAppSc Aqua, GDipAquaclt*

June 2013

Submitted in fulfillment for the degree of Master of Applied Science

National Centre for Marine Conservation and Resource Sustainability

AMC, University of Tasmania

Declaration of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of the my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

....

David Ellis Date

Authority of Access

I, the undersigned, author of this thesis, understand that the University of Tasmania/Australian Maritime College may make it available for use within the University Library and, by other means, allow access to users in other approved libraries. Copying and communication of any part of this thesis is prohibited for two years from the date this statement was signed; after that time limited photocopying and communication is prohibited in accordance with the Copyright Act 1968.

....

David Ellis Date

Statement of Ethical Conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University. The research methodology received clearance from the University of Tasmania Experimentation Ethics Review Committee (Approval number A0008195)

....

David Ellis Date

Statement of the contribution of other parties

Funding

- Aquafin CRC
- Fisheries Research & Development Corporation

Supervision

- Assoc Prof John Purser, University of Tasmania
- Prof Chris Carter, Tasmanian Fisheries Institute
- Prof Rob van Barneveld, Barneveld Nutrition

Research in-kind support

- Australian Southern Bluefin Tuna Industry Association

....

David Ellis Date

Acknowledgements

I wish to thank my supervisory team. Firstly I wish to thank Assoc Prof John Purser for his guidance, perseverance, and understanding of managing a challenging student. Prof Chris Carter for his high level physiology and nutrition guidance and Prof Rob van Barneveld for his practical approach to advising on trial design.

I'm not sure whether to thank Brian Jeffriess for highlighting this pathway and urging me to do it or curse him for the anguish and emotional scarring.

This research would not have been possible without the assistance of SBT ranching companies, their staff, and researchers over the past eon.

I appreciate the technology transfer, assistance and guidance provided by John Gunn, Jason Hartog, Naomi Clear and Toby Patterson from CSIRO. Special thanks to Kirsten Rough and Thor Carter who played a pivotal role in this research by educating me in the surgical procedure.

I am inspired by the commitment of Justin Nelligan and Rick Kolega - without you none of this research would have been possible. Other industry people including Craig Hughes, Adam Watkins, Stephan Schilling, and Adam Kayser provided invaluable support. Big thanks to my big mate David Warland for his passion towards this research. The contributions of SBT ranching companies: Blaslov Fishing, Sekol Farmed Tuna, DI Fishing and Kistuna are sincerely appreciated.

Researchers actively involved in this research in different capacities were Danielle Foote, Ron Denny, Teua Temaru, Kane Williams, Steven Clarke from SARDI, and Peter Montague from AquafinCRC. The statistics guidance from Megan van Depeer, Nicole Kirchoff, Mel Leef and Ronald Fischer is much appreciated.

To close friends and family, I sincerely appreciate your help, support and understanding.

For inspiration -

Look, if you had one shot, or one opportunity

To seize everything you ever wanted in one moment

Would you capture it or just let it slip?

Opening song lyrics from "Lose Yourself" by American hip-hop artist Eminem, released as the first single from the original soundtrack to the movie *8 Mile* on October 22, 2002

Dedication

This thesis is dedicated to

Ross Guerin and Albert Ellis

These blokes taught me many things and the older I get I recognise the real value of grandfathers. I only wish that they were still around to catch up with and share their life experiences.

Abstract

Southern bluefin tuna (*Thunnus maccoyii*) warm their viscera when digesting food. Through surgical implantation of archival tags, this thesis explores visceral warming patterns in southern bluefin tuna (SBT) with the aim of identifying relationships between visceral heat, nutrient supply, feed frequency and efficiency in SBT.

Based on six trials with different but related objectives, it was found that dietary energy influences visceral warming, time taken to reach peak visceral temperature and duration of visceral warming when SBT receive one meal per day. When SBT are fed more than one meal per day, feed intake may be measured when dietary energy is known and water temperatures are cool. Different industry feeding practices were shown to have no impact on visceral warming patterns when SBT were regularly fed two times per day with a high energy diet compared with six times per day using a low energy diet emphasising the importance of providing an appropriate protein and lipid balanced diet. SBT visceral warming patterns in this trial altered when regular meals were missed.

An 18 week trial involving four baitfish feeding treatments with different protein to lipid ratios fed in 3 x 6 week time periods demonstrated that maintaining a consistent feed profile of approximately 7 % lipid especially in the first 6 weeks of culture will optimise SBT performance in respect to growth, food conversion and body condition. Specific growth rates from all treatments were significantly better than Atlantic bluefin (*Thunnus thynnus*) of the same size and age. An analysis of dietary energy with regard to visceral warming showed that dietary energy is not a reliable measure of feed intake and that visceral warming is more influenced by water temperature and feeding behaviour. In cooler water temperatures SBT feed less, increase visceral warming and conserve heat. In warmer water temperatures SBT feed more and expend visceral heat suggesting that SBT have a physiology response to body temperature that is not directly related to dietary energy intake.

A trial investigating visceral and tissue temperature profiles showed that at water temperatures of 20^{o}C or less, SBT maintain basal and maximum visceral temperatures between 4^{o}C and 10^{o}C above ambient water temperature and that visceral temperatures can be predicted with confidence. At water temperatures above 20^{o}C the relationship

between basal and maximum visceral temperatures and water temperature may be predicted with less certainty. SBT maintain red muscle temperature at approximately 30^{o}C irrespective of feeding regime or water temperature, white muscle temperature at approximately 6^{o}C above water temperature irrespective of water temperature and feeding regime, but visceral cavity temperature of SBT is influenced by both water temperature and feeding regimes in water temperatures up to approximately 20^{o}C. Temperature profiles developed through this research suggest that water temperatures above 20^{o}C lead to heat stress in SBT.

List of Figures

List of Tables

List of Abbreviations

ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ATBOA	Tuna Boat Owners Association of Australia
AOAC	Association of Analytical Communities
CCSBT	Commission for the Conservation of Southern Bluefin Tuna
CSIRO	Commonwealth Scientific Industrial Research Organisation
DMS	Diesel Marine Services
FCR	Food Conversion Ratio
FRDC	Fisheries Research & Development Corporation
GET	Gut Evacuation Time
GG	Gilled and Gutted
GLM	General Linear Model
FD	Feed Duration
FI	Feed intake expressed as a % of body weight
FM	Feed Measure
FTE	Full Time Equivalent
HP/LL	High Protein Low Lipid
ITQs	Individual Transferable Quotas
Local	Sardines caught from waters surrounding Port Lincoln
LP/HL	Low Protein High Lipid
LSD	Least Square Difference
MH	Maximum Heat
MP/ML	Medium Protein Medium Lipid
OFCF	Overseas Fishery Cooperation Foundation
PIRSA	Primary Industries Resources South Australia
RAN	Royal Australian Navy
SBT	Southern Bluefin Tuna
SDA	Specific Dynamic Action
SGR	Specific Growth Rate

T_b	Basal visceral temperature
T_{max}	Maximum visceral temperature
T_{rm}	Red muscle temperature
T_{vis}	Visceral temperature
T_w	Water temperature
T_{wm}	White muscle temperature
T1	Treatment 1
T2	Treatment 2
T3	Treatment 3
T4	Treatment 4
t_{max}	Time to reach maximum visceral temp

Table of Contents

Chapter 1 - Introduction

1.1 Introduction

Southern bluefin tuna (*Thunnus maccoyii*)(SBT) are caught in the pristine waters of the Great Australian Bight on Australia's south coast using innovative fishing techniques developed by the fishermen of Port Lincoln and are ranched in the clear waters of Spencer Gulf near Port Lincoln (Jeffriess, 1999) (Figure 1.1.1).

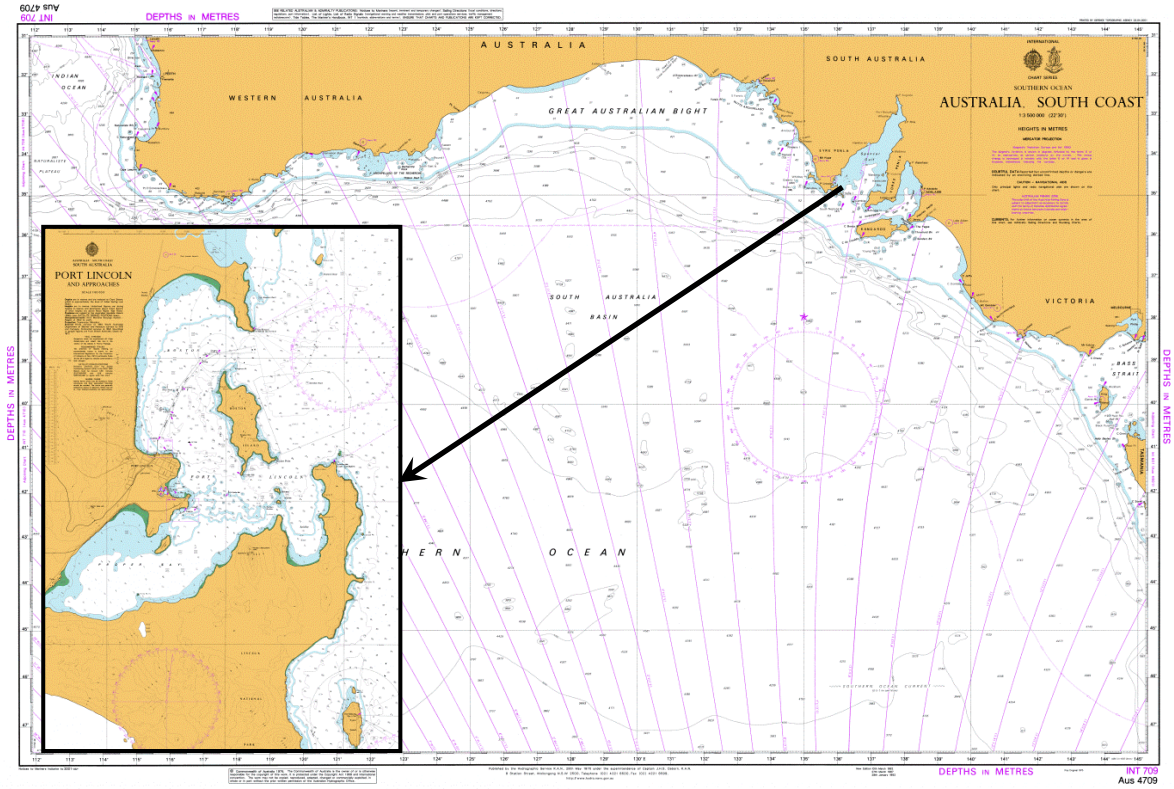


Figure 1.1.1 Geographic location of Port Lincoln, South Australia. Cadastral chart Aus 04709 of the Australian South Coast with insert of Aus 00134 Port Lincoln and Approaches (Calder, 1977).

The SBT industry is an Australian success story pioneered by the Port Lincoln fishing industry, and is the single most valuable sector of South Australia's aquaculture industry (PIRSA, 2012); however it has not been without its challenges. Since 1991 the industry has participated in two Cooperative Research Centres involving major Australian research institutions, investing a significant amount of money to ultimately understand, enhance and improve farming/ranching methods.

Whilst significant advances have been made in areas involving fish health, nutrition, product quality, physiology and metabolism and the environment (Montague et al., 2008) there is still a long way to go to fully optimise ranching operations.

The single biggest cost to SBT ranching operations is feed. Through the use of implanted archival tags in the visceral cavity this thesis will assess visceral warming patterns and explore SBT physiological response to feed. This thesis explores whether visceral warming can be useful in predicting the weight and nutritional content of feed ingested by ranched SBT in commercial applications. The results of this thesis will inform improvements in ranched SBT feeding practices and enhancements in SBT productivity and performance.

1.2 Aims and objectives

This thesis aims to explore the relationship between visceral heat change, nutrient supply, feed frequency and efficiency in SBT. It builds on existing research in this field and explores assumptions within previous nutrition studies.

The thesis is structured around six trials with the following objectives:

Trial 1 – The visceral warming response in southern bluefin tuna (Thunnus maccoyii) to a single meal with an emphasis on volume of feed ingested, dietary energy and baitfish size

The objectives of this Trial were to assess whether archival tags implanted into the visceral cavity of two to three year old free swimming SBT could record visceral warming patterns; and to test whether there are predictable patterns in the visceral warming response to feed intake measured by weight, baitfish size and nutritional energy of the feed ration offered. The research followed recommendations of Gunn et al. (2002) by investigating the influence of dietary energy on the measure and duration of visceral warming, and the time taken to reach peak visceral temperature.

Trial 2 – The visceral warming response to one, two or three feeds in southern bluefin tuna (Thunnus maccoyii) with an emphasis on weight of feed ingested and dietary energy at two different water temperatures

The objectives of this Trial were to assess whether archival tags implanted into the visceral cavity of two to three year old free swimming SBT could record visceral warming patterns;

to investigate weight of intake, dietary energy and feed frequency and the response to duration and time to reach peak temperature at two different water temperatures. Data obtained from Trial 1 and this Trial were combined to examine visceral warming patterns in relation to ambient water temperatures.

Trial 3 – The measurement of temperature in red muscle, white muscle and the visceral cavity in slaughtered southern bluefin tuna (Thunnus maccoyii) in response to three feeding regimes and ambient water temperature

The objectives of this Trial were to investigate the temperature profile of red and white muscle and viscera in response to three feeding regimes and ambient water temperature.

This Trial builds on previous research (Carey et al., 1984; Graham and Dickson, 2000; Sepulveda et al., 2007) by exploring regional endothermy and visceral warming in SBT, and the thermoregulation response of SBT in relation to water temperature and feeding (Graham and Dickson, 2000; Graham and Dickson, 2001; Dickson and Graham, 2004).

Trial 4 – The measurement of maximum and basal visceral temperature in commercially cultured southern bluefin tuna (Thunnus maccoyii) in response to commercial feeding practices at ambient water temperatures

The objectives of this Trial were to assess the response of commercial feeding practices on visceral warming patterns by using archival tags implanted into the visceral cavity of two to three year old free swimming SBT in response to ambient water temperature. Furthermore, the Trial will explore differences in physiology responses of SBT that had been tagged in the wild and SBT that had been tagged on arrival in the farming zone.

Trial 5 – Feed intake, FCR, growth and proximate composition of southern bluefin tuna (Thunnus maccoyii) fed four diets in three periods over an 18 week period: Can feed intake from visceral warming patterns be predicted?

The objectives of this Trial were to utilise *FORMU-BAIT©* feed optimisation software developed by an Aquafin-CRC project (van Barneveld and Ellis, 2007; van Barneveld et al., 2009) to formulate combinations of baitfish diets varying in protein and lipid content to optimise SBT growth and feed intake over the ranching season. The Trial investigated the response of feeding regimes on feed intake, growth, Food Conversion Ratio (FCR) and the nutrient proximate body composition of SBT.

The Trial also assessed visceral warming in response to diet consumed and examined whether feed intake can be determined from visceral warming patterns.

Trial 6 – Measurement of visceral warming patterns in commercially grown southern bluefin tuna (Thunnus maccoyii) in response to two feeding regimes

The objective of this Trial was to assess visceral warming patterns in response to feeding events. The Trial investigated whether SBT fed when offered feed and associated visceral warming responses. The Trial also examined different feeding regimes employed by industry throughout the culture season and the influence of adverse weather conditions that can restrict or prohibit feeding in the tuna farming offshore zone.

1.3 Background

The history of the Port Lincoln SBT industry is one of intrigue, exploitation, uncertainty, desperation, political influence and innovation. It began in 1936 when Stanley Fowler from the Commonwealth Scientific Industrial Research Organisation (CSIRO) initiated a survey to assess tuna stocks using a military plane and fishing vessels. The survey was undertaken to stimulate economic development as until this time the fishery was largely unappreciated or exploited (Serventy, 1956). The survey was interrupted during the years of World War II when the survey vessel F.R.V. *Warreen* was commissioned by the Royal Australian Navy (RAN).

At the end of the war the vessel was re-commissioned until 1951 when the RAN transferred the vessel back again. The SBT fishery survey was continued using the F.R.V. *Stanley Fowler*, and the first commercial SBT trolling fishery is believed to have started in 1949 (Serventy, 1956).

In the 1950s the South Australian government financially supported the building of the purse seine vessel the F.V. *Tacoma*, in Port Fairy, Victoria (Plevin, 2000), from where it made its way to Port Lincoln.

After a series of start-up issues, the F.V. *Tacoma* caught its first catch of 10 tonnes of SBT destined for the local cannery in the northern area of Boston Bay near Port Lincoln. Following this catch, the South Australian government initiated a survey of tuna fish stocks using the F.V. *Tacoma* and fishing expertise from the United States of America (Figure 1.3.1).

Some of the proud crew of the *Tacoma* mid-1950's. Left to Right: Keith Bellamy, Cris Jangaard, A. R. Haldane, L. J. Brown, E. Plevin. Cris Jangaard, an American fisherman, had been contracted by the S.A. Government to survey tuna resources.

Figure 1.3.1 The proud crew of the F.V. '*Tacoma'* on return from a South Australian Government funded survey of tuna resources in the Great Australian Bight. Note that only one of the six fish in the photograph is an SBT (Axel Stenross Museum, Port Lincoln)

The results of the survey initiated an expansion of the tuna fishing industry and vessels began to use the tuna-poling method, trolling and purse seine to haul in SBT catches. The 1960s and 1970s witnessed a rapid increase in SBT fishing with record catches from fishing pole fishing, long-line and purse seine gear in the unregulated fishery (Figure 1.3.2).

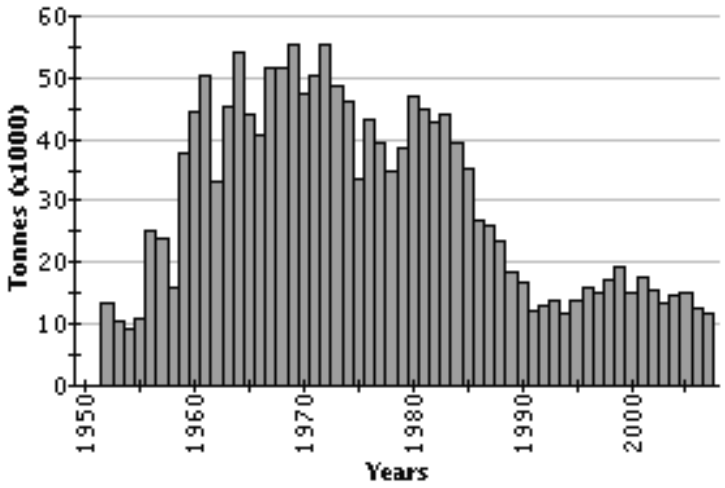


Figure 1.3.2 Global catches of SBT 1950 to 2010 (FAO Fishery Statistic)

Spotting planes were introduced to locate schools of SBT from the air and direct vessels to their location. Vessels changed from wood to metal and fishing effort increased through the cooperation between purse-seine and pole-fishing vessels (Campbell, 2001) resulting in significantly more SBT landings. The catches underpinned the local economy primarily through employment in the Port Lincoln tuna cannery. However, in 1979 fishery biologists warned that the fishery was fully exploited, the parental biomass was being reduced at an alarming rate (30% of its pre-exploitation size), and that this would ultimately result in poor recruitment of juvenile SBT to the fishery in subsequent years. Despite these warnings, fishing effort was maintained and the Australian catch reached a peak of 21,000 tonnes in 1982 (Geen and Nayer, 1989).

The fishing pressure/effort continued into the early 1980s when an Australian Government inquiry in 1983 found that the fishery was biologically over-exploited and heavily over-capitalised (Geen and Nayer, 1989). In the meantime, fishing vessels were scouring the ocean for ever scarcer schools of SBT.

In 1984 Individual Transferable Quotas (ITQs) were handed out by the Australian Government to fishermen to prevent any further exploitation and growth in the industry. Japanese and New Zealand governments also agreed to limit catches. As SBT catch was significantly below quotas set by Australian, Japanese and New Zealand governments between 1984 – 1988, all three countries agreed to further reduce catch limits with annual reviews (Geen and Nayer, 1989).

Informal management of the SBT fishery between the three countries was formalised in 1994 through the formation of the Commission for the Conservation of Southern Bluefin Tuna (CCSBT). Today there are seven member countries of the CCSBT (Anon, 2012).

In 1989 a trilateral conference was held between Japan, Australia and New Zealand where it was agreed that the total combined yearly quota for all three countries would be limited to 11,750 tonnes. With this in mind It became very clear to the fishermen of Port Lincoln that to survive financially in the face of fishing restrictions and over capitalisation that they had to increase the value of their fishery.

The first approach was an unsuccessful fishing venture into the Pacific Ocean with super seiners to catch skipjack tuna for the canning market. On the back of this failed venture, the industry began to investigate options for increasing SBT yield through tuna farming with the lucrative Japanese sashimi market in mind (B Jeffriess 2011, pers comm., 14 December).

A study was initiated by the Tuna Boat Owners Association of Australia (ATBOA) and the Federation of Japan Tuna Fisheries Co-operative Associations in conjunction with the Overseas Fishery Cooperation Foundation (OFCF) and the support of the South Australian Government and the Australian Government (Anon, 1993). This project was undertaken by the SBT industry in partnership with the Fisheries Research and Development Corporation (Anon, 1993; Jeffriess, 1999).

The value of the SBT on the Japanese sashimi market was quickly realised and on the basis of this study, the industry progressed from poling individual SBT into vessel tanks to the purse seine capture of schools of SBT transferred into specially designed towing pontoons and towed back to Port Lincoln for further for on-growing in static ranching pontoons. This change in catching approach facilitated the rapid expansion and development of the Port Lincoln tuna ranching industry.

Since 1990, the industry has steadily expanded to produce up to 9,000 tonnes of gilled and gutted SBT annually with an estimated annual value of between AUD$150 - AUD$300 million (PIRSA, 2012). Direct and indirect employment in Port Lincoln is over 1500 FTE (Econsearch, 2007).

1.4 Southern bluefin tuna bio-physiology

The southern bluefin tuna (*T. maccoyii*) taxonomic position is in the Class Osteichthyes, Subclass Actinopterygii, Order Perciformes, Suborder Scombroidei and Family Scombridae which includes all mackerels and tunas (Lagler et al., 1977). The specific name *maccoyii* was bestowed by Castelnau (1872) with the comment that "the flesh of this fish is not eaten, or at least is not esteemed as food".

SBT are one of the largest bony fishes, living up to 40 years, growing to a length of 2.25 metres, and weighing over 200 kg (Patterson et al., 2009; Patterson et al., 2010). One specimen that washed up on a beach at Glenelg, South Australia in 1890 was reported to have weighed over 350 kg (Serventy, 1956).

SBT are a single, highly migratory stock (Patterson et al., 2009; Patterson et al., 2010) that is mainly found between the latitudes of 30° and 50°S (Collette and Nauen, 1983). The only known SBT spawning ground is located in the warm oceanic waters south of Java in the north-east Indian Ocean 10°-20°S, 105°-120°E, with the spawning season spanning September to April. Following spawning, the developing juveniles are transported in the Leeuwin Current along Australia's north western shores to the south west tip of Australia and into the Great Australian Bight or west towards South Africa (Figure 1.4.1) (Campbell, 2001; Patterson et al., 2009). There is some uncertainty about when SBT reach spawning age but the general view is that it is between 8 and 12 years with females producing several million eggs in a spawning period (Hayes, 1997). SBT are opportunistic feeders (Dickson, 1996), preying on fish, crustaceans, cephalopods, salps, and other marine animals (Young et al., 1996; Young et al., 1997; Itoh et al., 2011).

Tuna have a range of distinguishing anatomical and physiological adaptations that assist with movement to minimise anterior resistance and maximise caudal thrust (Bushnell and Jones, 1994; Dewar and Graham, 1994; Brill, 1996; Fitzgibbon et al., 2008). These adaptations include a streamlined shape that is built for speed, maneuverability, drag reduction and efficiencies in locomotion (Magnuson, 1978; Dewar and Graham, 1994).

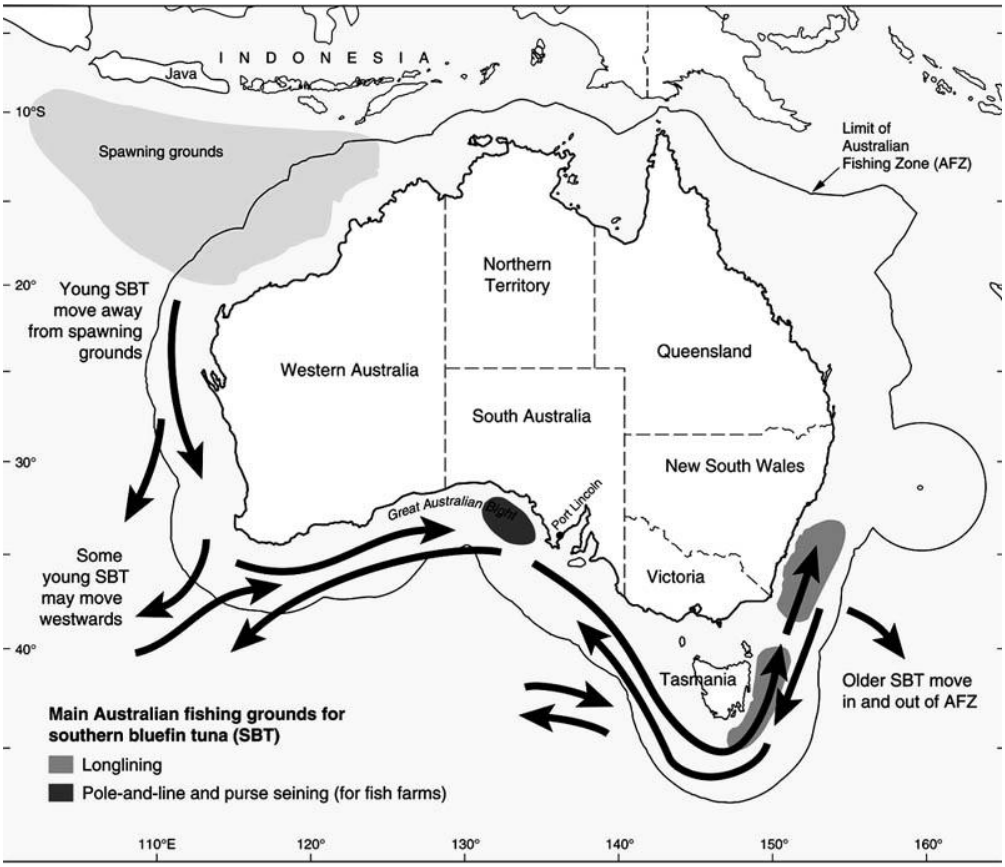


Figure 1.4.1 Geographic range of the Australian southern bluefin tuna fishery (Campbell, 2001).

Their dorsal, pelvic and pectoral fins provide guidance but serve no role in propulsion. When moving at high speed (approx 70 km/h) (Wardle et. al., 1989) SBT use long propulsive beats of their tail and retract their fins into defined body grooves to minimise drag. Caudal keels along the top and bottom edges of the body act as spoilers to prevent turbulence (Attenborough, 1979; Altringham and Shadwick, 2001).

Tuna have been described as energy speculators because they "gamble" high rates of energy expenditure in nutrient poor pelagic environments for the capture of prey. This hunting approach depends on being able to capture and process food as efficiently as possible (Korsmeyer et al., 1996).

Similar to a number of other ocean roaming predators including lamnid sharks, billfishes, and the opah (*Lampris guttatus*) (Block et al., 1993; Block and Finnerty, 1994; Dickson and Graham, 2004; Runcie et al., 2009), some tuna are able to warm their viscera, myotomal muscles, eyes and brain above ambient water temperature, a characteristic known as regional endothermy (Carey et al., 1984; Graham and Dickson, 2000; Graham and Dickson, 2001; Dickson and Graham, 2004; Sepulveda et al., 2007).

Development of endothermy varies considerably within the *Thunnini* tribe and correlates strongly with tuna phylogeny (Block et al., 1993; Collette et al., 2001). However, only species within the subgenus *Thunnus*, including southern bluefin tuna (*T. maccoyii*), Atlantic bluefin (*Thunnus thynnus*), Pacific bluefin (*Thunnus orientalis*), albacore (*Thunnus alalunga*) and bigeye tuna (*Thunnus obesus*) are capable of visceral endothermy (Collette et al., 2001).

The thermal biology of tuna is well documented (Laurs et al., 1977; Olson and Scholey, 1990; Dewar and Graham, 1994; Dickson, 1994; Altringham and Block, 1997; Korsmeyer et al., 1997a, Korsmeyer et al., 1997b). Tissues capable of being warmed include myotomal muscles, eyes and brain, and the viscera (Graham and Dickson 2000; Graham and Dickson 2001; Dickson and Graham, 2004). Large Atlantic bluefin tuna achieve visceral temperatures of up to 21^{o}C and can maintain body temperature of 8^{o}C to 15^{o}C above ambient water temperature for months (Carey, 1973; Carey et al., 1984; Block et al., 2001; Gunn and Block, 2001).

Smaller southern and Pacific bluefin tuna maintain visceral temperatures between 3^{o}C to 9^{o}C above ambient water temperature (Kitagawa et al., 2000; Gunn et al., 2001; Itoh et al., 2003; Kitagawa et al., 2006). Research on SBT weighing between 15.5 kg and 39.6 kg has demonstrated that SBT maintain their basal visceral temperature approximately 2^{o}C above ambient water temperature (Gunn et al., 2002). Maximum visceral temperatures up to 32^{o}C have been reported in summer, and maximum visceral temperatures between 26^{o}C and 27^{o}C have been recorded in winter (Gunn et al., 2002).

Regional endothermy enables tunas to expand their thermal niche from surface waters to depths greater than 400 m, and to migrate large distances (Block et al., 1993; Graham and Dickson, 2000; Dickson and Graham, 2004). It improves the performance of tuna, enhances rates of muscle contraction and power output (Altringham and Block 1997), cellular respiration (Stevens and Carey, 1981), vision, neural processing (Block and Carey, 1985), and digestion (Carey et al., 1984; Gunn et al., 2002).

Endothermy requires a source of heat and a mechanism to retain it. Tuna are believed to digest food more rapidly than other fish (Magnuson, 1978; Olsen and Boggs, 1986; Brill, 1996), and the heat source for endothermy is understood to be a by-product of their normal metabolic processes.

Visceral warming is driven by Specific Dynamic Action (SDA) which refers to the total energy cost associated with ingestion, digestion, absorption and assimilation of food. All fishes generate "waste" heat from metabolic processes including that associated with muscle contraction and SDA related processes (Graham and Dickson, 2001). However in most fishes waste heat is lost through convective heat transport through the gills.

In SBT, it is estimated that 35% of ingested energy may be attributed to SDA (Fitzgibbon et al., 2008). In contrast, only 14.19 ± 4.19 % of ingested energy may be attributed to SDA in freshwater fish such as largemouth bass (*Micropterus salmoides*) (Beamish, 1974), and up to 23% of ingested energy may be attributed to SDA in grass carp (*Ctenopharyngodon idella*) (Carter and Brafield, 1992). Heat generated through digestion and mechanical energy used to capture and metabolise food may also contribute to visceral warming (Gunn et al., 2002). A typical SBT visceral heating pattern (Gunn et al., 2002) is very similar to that of Atlantic bluefin (*T. thynnus*) as described by Carey et al., (1984). The increase in visceral temperature results in increased heat production and efficiency of heat conservation as a direct result of the hydrolytic breakdown of food. The heat generated through these sources is retained within tissues through counter current heat exchange (retia mirabilia) via arterial and venous blood vessels arranged to reduce convective heat loss (Collette, 1978; Carey et al., 1984; Fudge and Stevens, 1996; Graham and Dickson, 2001). Tuna viscera temperatures have been observed to be significantly different to ambient water temperatures before, during and after digestion of food (Carey et al., 1984).

Visceral temperatures and metabolic rates rise abruptly after feeding and then decline steadily, with the magnitude (total increase above basal level) of postprandial metabolic increment and postprandial visceral warming being proportional to the amount of food ingested (Carey et al., 1984; Gunn and Block, 2001; Gunn et al., 2001; Gunn et al., 2002; Fitzgibbon et al., 2008). A return to basal temperatures usually occurs between 36 h to 48 h after feeding (Gunn et al., 2002). Trials on Atlantic bluefin tuna (*T. thynnus*) have shown basal temperatures between 3^{o}C to 6^{o}C above ambient water temperature, and temperatures between 10^{o}C to 15^{o}C above ambient water temperature after consumption of food (Carey et al., 1984). It has been demonstrated that Southern bluefin tuna (*T. maccoyii*) maintain basal visceral temperatures 2-4 ^{o}C above ambient seawater temperature (Gunn et al., 2002).

1.5 Rationale

There is limited information available on the nutritional requirements of SBT during their growing phase within a ranching environment. Information that is available tends to focus on the use of manufactured diets (Clarke et al., 1997; Carter et al., 1998; Glencross et al., 1999; van Barneveld et al., 1999; Glencross et al., 2002; Gordon et al., 2006a; Gordon et al., 2006b; van Barneveld and Ellis, 2007; van Barneveld et al., 2009), and little attention has been given to feeding efficiencies with regard to baitfish (Gunn et al., 2002; Ellis and Rough, 2005; van Barneveld and Ellis, 2007).

Current practices involve the feeding of a mixture of baitfish to SBT within pontoons (Ellis and Rough, 2005) until they are ready for harvest which could be from two to six months. While industry has attempted to replace baitfish with manufactured diets (Clarke et al., 1997; Carter et al., 1998; Glencross et al., 1999; van Barneveld et al., 1999; Glencross et al., 2002; Gordon et al., 2006a; Gordon et al., 2006b; van Barneveld and Ellis, 2007; van Barneveld et al., 2009) there have been problems associated with weaning SBT to a foreign food source, the form of the diet, ingredients and cost (van Barneveld and Ellis, 2007).

Research into nutrition of ranched SBT must take account of a number of parameters that currently constrain production. In general, parameters include the considerable cost of feeding (Vega et al., 1994), nutrient supply and delivery, and minimisation of waste.

No one feed ingredient is likely to be able to supply fish with all required nutrients (Ruohonen et al., 2007) so that the design and delivery of optimised diets requires consideration of cost effective options that offer efficient nutrient supply relative to nutritional requirements (Glencross et al., 1999).

In general, farmed fish are not provided with food that is made of their natural prey. Instead, feed is manufactured from a range of raw ingredients that include essential and non-essential nutrients that are aimed at meeting the animal's nutrient requirements for optimal growth and performance (Jobling, 2001). Feed intake is critical to any assessment of the relationship between diet composition and performance (McCarthy et al., 1993; Sugiura, 2000).

Assessment of feed intake is significantly influenced by holding facilities (Lazo and Davis, 2000), feeding rhythms (Madrid et al., 2001), and research methods (Thodesen et al., 1999;

Begout Anras et al., 2001; Jobling et al., 2001; Ruohonen et al., 2001; Mazlan and Grove, 2003; Bestley et al., 2008; Bestley et al., 2010). Feed intake may also change between days (Juell et al., 1994) and seasons (Jobling and Baardvik, 1991), as well as water temperature (Jobling, 1994; Peres and Oliva-Teles, 2001). It has been shown in SBT that feed intake changes significantly as water temperatures drop (Glencross et al., 2002) and as SBT gain condition expressed as body mass to body length^3 metric (van Barneveld and Ellis, 2007).

Methods used to measure feed intake in fish include stomach contents analysis (Hamre et al., 2001), introduction of tracers in the diet including dyes and chemical markers (Sigurgisladottir et al., 1992; Morales et al., 1999; Ward et al., 2005; Martins et al., 2009) and subsequent collection of faeces (Storebakken and Austreng, 1988; Lazo and Davis, 2000; Vandenberg and De La Noüe, 2001; Carter et al., 2003), direct observation and video recording (Ang and Petrell, 1997), demand feeders (Yamamoto et al., 2002) with waste collection and monitoring, x-radiography (Mazlan and Grove, 2003) and introduction of dense particles such as small glass beads in the feed (McCarthy et al., 1993; Jobling et al., 2001). While many of these techniques have been applied to SBT nutrition research, their success has been limited (van Barneveld et al., 1999; van Barneveld and Ellis, 2007). The measuring of feed intake in ranched SBT is a priority with regards to improving information on nutritional requirements.

Baitfish is still the primary source of feed for ranched SBT. There over 20 species of domestic and internationally caught baitfish used in the industry (Ellis and Rough, 2005; van Barneveld and Ellis, 2007) although the locally caught sardine (*Sardinops sagax*) accounts for over 50% of the industry feed requirement. The baitfish used are variable in size and proximate nutritional content ranging from <1% to 20% lipid and 13.3% to 20.1% protein (Ellis and Rough, 2005). Evidence shows that an individual SBT can consume an average of up to 3 kg of baitfish per day during peak feeding times approximately 10% of body weight. However, a SBT fed manufactured feeds during the same time will only peak at 1.8 kg per day (van Barneveld and Ellis, 2007). It is hypothesised that the reduced consumption of manufactured feed may be due to the binding of the diets, feeding regimes, absorption of nutrients, or the fat coating on manufactured diets (van Barneveld and Ellis, 2007).

Anecdotal evidence suggests that SBT farmers have been blending baitfish supplies for some time in an attempt to improve fish growth and product quality, the mix of baitfish being

determined by the 'gut feel' of the farmer. Some farmers prefer to feed high lipid diets early in the season when feed intake is high to condition SBT for early marketing opportunities and to 'pack the weight on'. Some farmers believe that SBT should be fed with low lipid/high protein baitfish blends to increase the length of the SBT before switching to a high lipid diet later in the season to condition the SBT in the last phase of the growout cycle.

Other farmers prefer to feed a consistent medium protein /medium lipid diet for the duration of the season, and some companies own sardine fishing vessels and only use local sardines for feed.

The composition of a diet is important to fish performance and health. High lipid feeding regimes have led to increased adiposity in turbot (*Scophthalmus maximus*) (Saether and Jobling, 2001), and lipostatic regulation of feed intake in Atlantic salmon (*Salmo salar*) (Johansen et al., 2001; Johansen et al., 2002) and juvenile ayu (*Plecoglossus altivelis*) (Lee et al., 2002). Feed intake has been reported to increase in European sea bass (*Dicentrarchus labrax*) when diets are high (30 %) in lipid (Peres and Oliva-Teles, 2001), but when high lipid diets are fed to Polka-dot groper (*Cromileptes altevelis*) they demonstrate depressed performance (Williams et al., 2006). In European whitefish (*Coregonus lavaretus*) elevated flesh fattiness is a consequence of over-consumption of lipids (Ruohonen et al., 2007). However, there is little information available on the optimal blend of baitfish to optimise growth, health and production of SBT.

Feeding in SBT aquaculture operations tends to occur during the daylight hours and to match the natural feeding behaviour of diurnal feeding fish (Eriksson and Alanärä, 1992; Bolliet et al., 2001). Many aquaculture fish display rhythmic feeding patterns when access to feed is uninhibited and often change their feeding preferences during different times of the year (Smith et al., 1993; Blyth et al., 1999). Daily and seasonal changes may reflect nutrient requirements and it is possible that metabolism is not constant and that there is seasonal dependency (Bolliet et al., 2001).

It has been demonstrated that feeding frequency and the delivery of feed rations can impact on food conversion efficiencies and consequent weight and length gain (Boujard and Leatherland, 1992; Jarboe and Grant, 1996). For example, juvenile olive flounder (*Paralichthys olivaceus*) grow better when offered three feeds per day to satiation (Lee and

Pham, 2010). African catfish (*Heterobranchus longifilis*) show optimal growth when fed continuously (Kerdchuen and Legendre, 1991), but Black Sea trout (*Salmo trutta labrax* Pallas, 1811) demonstrate a better food conversion ratio when fed once per day (Bascinarl et al., 2007). Asian sea bass (*Lates calcarifer*) grow best on two feeds per day (Salama, 2008). Some studies demonstrate that nocturnal feeding eliminates density dependent growth suppression (Jørgenson and Jobling, 1993) and results in higher growth rates (Reddy et al., 1994).

It is assumed that, in any case, feeding fish in time with their natural rhythms may result in optimal nutrient utilisation (Bolliet et al., 2001).

The timing and delivery of an optimised diet can also have a significant financial impact on SBT ranching operations due to their geographical location. SBT feed frequency research has demonstrated that when SBT are fed twice a day to satiation four, five or six times per week in summer, there is a significant negative growth difference in the four day feeding regime compared with the five and six day feeding regime (Gunn et al., 2002). In other words, SBT fed five or six days per week grew better than those fed four days per week. Given the substantial costs associated with feeding ranched SBT, improvements in feed frequency and delivery are of considerable interest to SBT farmers.

1.6 Limitations

There are significant challenges to undertaking research on ranched SBT due to the physiology of SBT and the difficulties experienced in working with tunas in general (Korsmeyer and Dewar, 2001; Clark et al., 2008). As a result, it has been necessary for specific handling techniques to be developed to enable research and minimise stress on the fish (Carter et al., 1998; Musgrove and Fitzgibbon, 2006; Thomas, 2007; Hutchison et al., 2008; Thomas et al., 2009; van Barneveld et al., 2009; Nowak et al., 2010). For example, there are a range of physiological factors that need to be considered when placing SBT into pontoons or crowding them. Tuna are obligate ram ventilators in that they need to swim to maintain water flow over the gills for oxygen as they do not have the ability to rest completely and pump water through their gills like other fish (Magnuson, 1978; Korsmeyer and Dewar, 2001). Should SBT become entangled or stunned they will often asphyxiate and die.

Research design and outcomes can be influenced by the health and condition of wild caught SBT prior to stocking in static ranching pontoons (Nowak et al., 2010), the mixture of cohorts within captured schools (Gunn et al., 2002; van Barneveld et al., 2009), and stress associated with the stocking density of the towing pontoon (Nowak et al., 2010), extreme weather events such as lightning and water conditions (K Rough 2012, pers. comm., 20 January), impacts of predators such as sharks and pinnipeds (M Thyer 2012, pers. comm., 20 January) and killer whales (Guinet et al., 2007).

Research design is constrained by the cost of studying SBT. Commercial constraints and business operations tend to restrict access to fish and therefore have a significant impact on research methods (Carey et al., 1984; Aguado-Gimenéz et al., 2006). Costly, specialised infrastructure, vessels and research personnel are also required to undertake SBT research.

The results of SBT research are also influenced by the environments within which the research is undertaken. For example, research that involves the ranching of SBT in smaller pontoons often requires the pontoons to be located in a protected environment with different environmental conditions to that of commercially offshore grown SBT.

Due to these limitations, SBT research requires innovative approaches (Carter et al., 1999) such as the use of surrogate species to investigate nutrition (Clarke and Ham, 2002), application of a mesocosm respirator chamber to understand SBT metabolism and physiology (Fitzgibbon et al., 2006; Fitzgibbon et al., 2007; Fitzgibbon et al., 2008), and an impervious pontoon liner with a lift-up system to facilitate ingredient digestibility studies (Hutchison et al., 2008), and archival tags (Gunn et al., 2002).

CSIRO adapted biological tags and created archival tags suitable for free roaming SBT in 1993 to improve information on their physiology (Gunn et al., 1994). An archival tag consists of a base module incorporated into a micro chip board that is housed in resin to protect the electronic components. Tags were first implanted in wild SBT in 1994 (Gunn et al., 1994). The tags can record internal and external temperatures, depth and light intensity, and therefore enable detection of SBT migratory behaviours, swimming and diving behaviour, and visceral warming and cooling patterns (Gunn et al., 1994; Bestley et al., 2008; Bestley et al., 2010). Archival tags also present an opportunity to unlock information on the physiology and feeding behaviour of ranched SBT and thereby facilitating improvements to husbandry practices in SBT ranching operations.

1.7 Thesis structure

This chapter has presented background information on the southern bluefin tuna fishing industry and tuna bio-physiology.

The research aims and trial objectives are described and the rationale for the research program is detailed. The next chapter presents information on general methods used across all of the six Trials that form part of this research, as well as identifying specific methods applied in individual Trials. Chapter 3 presents a description of the research undertaken for each individual Trial, and Chapter 4 outlines research results including visceral warming patterns and influences, regional endothermy and SBT performance in the context of available data on tuna and other aquacultured finfish. Chapter 5 presents a synopsis of results in relation to the research aims and implications for industry feeding practices, and describes recommendations for future research in this field.

Chapter 2 - Methods

This chapter presents the methods used to address research aims and objectives. General methods used for all six Trials are described, including fishing capture methods, the surgical implantation and removal of archival tags, and approaches towards analysis and interpretation of data obtained through the archival tags. Specific methods used in each Trial are also detailed.

2.1 Methods used in all trials

The methods described in this section were applied across all six of the Trials on which this thesis is based.

SBT Capture

The 2 to 3 year old SBT used in this research were caught by commercial purse-seine vessels in the Great Australian Bight in a wide-ranging area around -33^{o} 19' and 131^{o} 55' (Figure 2.1.1) during the Australian summer.

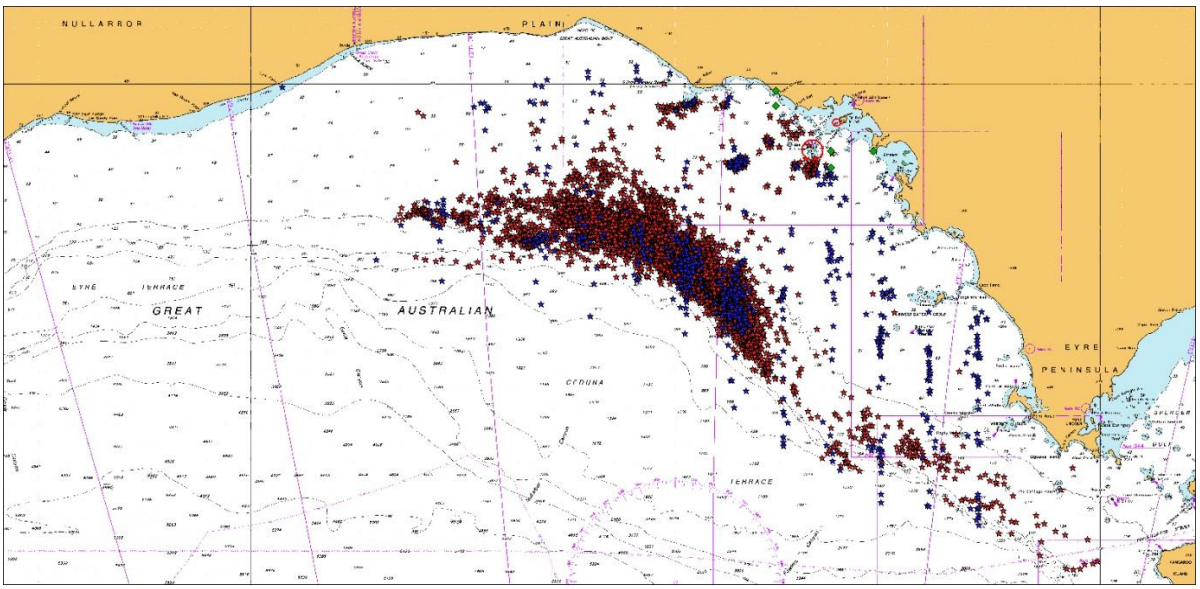


Figure 2.1.1 SBT school locations recorded by commercial SBT spotting planes and CSIRO aerial surveys 2000 – 2006. Red stars show SBT schools located by commercial spotting planes and blue stars show SBT schools located by the CSIRO aerial survey. Green diamonds are recreational SBT fishing areas.

Prior to being caught, schools of SBT were identified by a spotting plane which directed a chum vessel to the location of the school. On arrival, chum vessel deckhands cast thawed and live local baitfish (*S. sagax*) into the water, and in this way led the school of SBT to a position where a tow vessel and specially designed 45 m diameter towing pontoon (DMS, Port Lincoln Australia) were located. Once the SBT school was in close proximity to the towing pontoon, a commercial purse seine vessel encircled the chum vessel and school of SBT with a purse seine net measuring 1000 m long by 130 m deep. The purse seine vessel then retrieved the purse cable that runs along the bottom of the net resulting in the chum vessel and SBT school being contained in a net 'bowl'. Before the net was retrieved by the purse seine vessel, the chum vessel left the net enclosure. Smaller vessels powered by outboard engines kept the net open while the purse seine net was hauled on board and at the same time the tow pontoon was towed to meet the purse seine net containing the SBT (Figure 2.1.2).

Figure 2.1.2 Purse seine vessel F.V. *Joseph BJ* with majority of net on board waiting for tow pontoon to come alongside while small vessels hold the net open. Photo courtesy of Leith Whitakker.

Once the tow net (150 mm Badinotti 450 ply net, 45 m in diameter with 12 m walls) and the purse seine net were next to each other they were connected via a specially designed underwater gate by commercially trained divers. The purse seine vessel continued to haul the purse seine net on board which reduced the area for the SBT to swim in to the point where they swam through the underwater gate from the purse seine net into the tow pontoon net. After sufficient quantities of SBT had been caught and transferred into the tow pontoon the fish were transported back to Port Lincoln on a journey that could last up to three weeks. The SBT were stocked in the tow pontoon at a density of between 5 – 10 kg/m^3 and fed baitfish when weather and sea conditions permitted.

Insertion of archival tags

Animal ethics

Animal ethics approval for this research was obtained from the University of Tasmania under reference number A0008195.

Catching Procedure – insertion of conventional dart/archival tags

SBT to be inserted with conventional dart/archival tags were caught from the static ranching pontoon using a baited barbless hook size 8/0 (Diamond – Yamamoto, Japan) and handline. Once hooked, the SBT was drawn into a specially designed aluminium fish cradle and a wet dark cloth placed over its eyes to calm it and hook removed. The length of the SBT was measured using a scale bar in the cradle (±5 mm) and the weight recorded using a Salter clock face scale model 235 6S (± 200 g), suspended from a Hiab - hydraulic lifting arm (Hiab, Australia).

Surgical Procedure – insertion of conventional dart tags

While in the specially designed aluminium fish cradle, a conventional dart tag with a unique identifier (as used in tag and recapture studies by CSIRO and the CCSBT) was inserted into the SBT for identification (Hallprint, Australia).

Insertion of each tag involved retrieval of the tag from the supplied tag holder, creation of an arc in the tag with the forefinger and thumb to ensure that the tag remained inside the 4

mm tag applicator, and insertion into the SBT. The tag and applicator were disinfected in a solution of isopropanol alcohol or Betadine (Betadine, Australia).

The tag was inserted 1 cm forward of the anterior base of the second dorsal fin with the barb perpendicular to the dorsal spines. The tag was pushed through the pterygiophores towards the anterior end of the SBT and then rotated 90^{o} and removed from the SBT locking the tag in situ.

Archival tags

DST-Centi tags (Star-Oddi, Iceland), Minilog 12 bit tags (Vemco, Canada), and MK 9 tags (Wildlife Computers, Washington USA) were used in this research (Figure 2.1.3). Archival tags were preprogrammed to record temperature every four minutes.

Figure 2.1.3 Archival tags used in this study. From left: Star Oddi DST Centi; Vemco Minilog 12bit tag; and MK9 Wildlife tag.

Figure 2.1.4 A southern bluefin tuna on an operating mattress. Note dark coloured cloth over the head of the SBT. Placing the SBT upside down and covering the eyes has a subduing and calming effect on the SBT.

Preparing the SBT for surgical procedure

The SBT was rolled from the aluminium cradle coming onto a moistened vinyl lined foam operating mattress. To calm the SBT, it was placed upside down, a dark coloured wet cloth was placed over the eyes and the gill operculum was lightly tapped as a distraction (Figure 2.1.4).

Surgical Procedure

The surgical procedure has been described in Clark et al (2008). A 5 to 8 cm incision was made with a disinfected sharp paring knife in the belly wall along the ventral midline where two faint lines from the pelvic fins intercept. The incision point was approximately 10 to 12 cm anterior to the vent depending on the size of the SBT, and was made in an anterior to posterior direction to a depth that was in close proximity to the peritoneal cavity. The technician inserted a sterilised finger through the open incision and punctured the peritoneal cavity.

A 2.5 ml dose of broad spectrum antibiotic *Amoxil* (GlaxoSmithKline, Australia) was injected into the opening and the disinfected archival tag (Betadine, Australia) inserted in a downward motion to rest between the peritoneal wall, stomach and the pyloric caeca. Every attempt was made to ensure that the position of each archival tag inserted into SBT involved in this research was consistent with the thermistor resting between the pyloric caeca and middle of the stomach wall. The incision was then closed with 1 or 2 stitches of Vicryl suture size 1-0 (Ethicon, California USA). The SBT was raised from the operating mattress and introduced to the water upside down to ensure that water did not enter the wound under the pressure of entry. Once in the water the SBT righted itself and swam normally. The duration of the procedure from capture to the return of the SBT to the water was no more than two minutes.

Harvesting procedure

The SBT in the pontoon were crowded into a smaller area using a small purse seine net. Snorkelers entered the small purse seine net and captured the swimming SBT by grasping the caudal peduncle and pulling the SBT backwards in the water and at the same time gripping the operculum and rolling the SBT upside down. This process stunned the SBT at which point it was passed to a person on the harvest platform who directed a metal spike through the brain and into the base of the spine. The SBT was lifted from the harvest platform to the harvest table on the deck of the vessel. The brain was removed using a metal coring tool and a fibreglass rod was passed down the spinal column to destroy all spinal nerves. The SBT was weighed using Salter clock face scale; model 235 6S (± 200 g) and length measured with the tagging cradle (± 5 mm). The conventional dart tag was checked to align the numbering sequence with treatment and determine whether an archival tag had been placed inside the SBT.

The SBT was exsanguinated by making a 2 cm long x 2 cm deep incision using a small triangular knife through the dorsal ridge of the cavity that the pectoral fins fold back into during periods of high propulsion swimming, and into the main artery and vein (that run anterior-posterior found on either side of the SBT in the red muscle).

Following bleeding, an incision was made through the belly wall just in front of the vent and the intestine was pulled through the opening and severed.

The gill operculum was opened; a small cut was made behind the back of the eye and the operculum was pushed forward to open the brachial cavity. A single cut was made in a sweeping motion from the dorsal part of the gill basket where it meets the body of the SBT to the bottom of the gill basket from the lower jaw, severing connective tissue along the way. This process was repeated on the other side of the SBT and, in a twisting motion, the entire viscera were removed from the visceral cavity leaving the kidney. If an archival tag had been inserted into the SBT it was then recovered from the viscera, cleaned and downloaded using factory supplied software. Following these procedures, the SBT had a tag placed on the caudal peduncle for identification purposes, was placed into ice slurry, and transported to the processing factory for weighing if conditions on the vessel were too rough.

Measuring visceral warming patterns

Downloaded tags were analysed using *Archtag* analysis software (CSIRO, Australia).
Feeding events were measured by holding the left mouse button down at the location where the temperature fell below basal temperature (T_b), indicating that the SBT had consumed a meal. Whilst holding the mouse button down, the cursor was moved across the feeding event. A feeding event was defined by a sharp drop in temperature, followed by an abrupt rise in temperature, before steadily falling towards T_b. The mouse button was released at the point where the visceral warming pattern reached T_b (Gunn et al., 2002).

The recorded feeding event is shown in Figure 2.1.5. The outputs from the software on each feed assessment were as follows:

a) Feed Measure (**FM)** – this is a measurement of the visceral warming pattern (shaded area)**.** The calculation of the visceral warming pattern can best be explained as breaking the visceral warming pattern in to trapezoidal areas and then measuring the sum of each for a total as shown in Figure 2.1.6. The initial cooling is added to the Feed Measure(Gunn et al., 2002).
b) Feed Duration (**FD)** – this was the time measured from when the SBT consumed a meal until the viscera had returned to T_b (total time in minutes 1492 in Figure 2.1.5)**.**
c) Maximum heat (**MH)** – maximum in temperature increase above basal.

d) Time max (t_{max}) – time taken expressed as minutes to reach maximum visceral temperature.

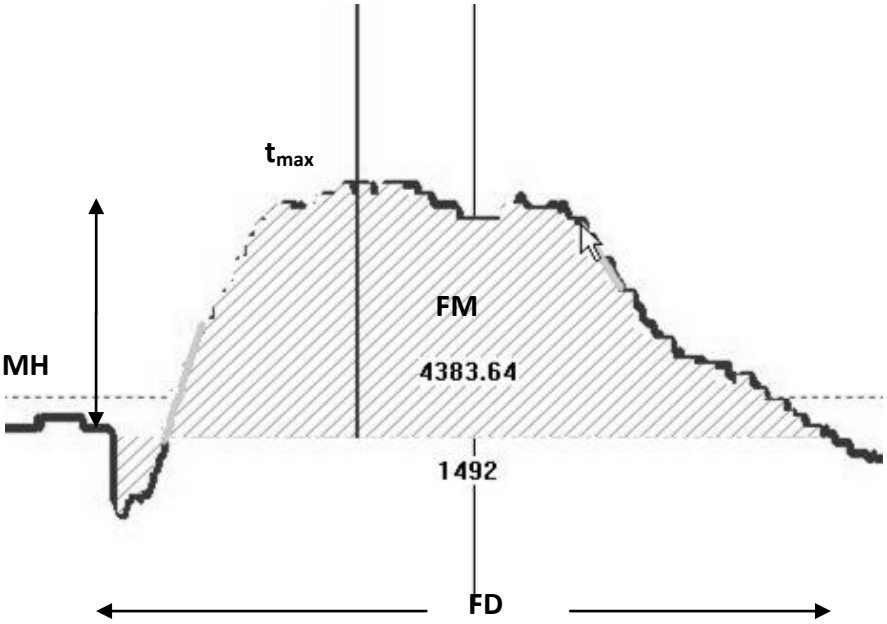
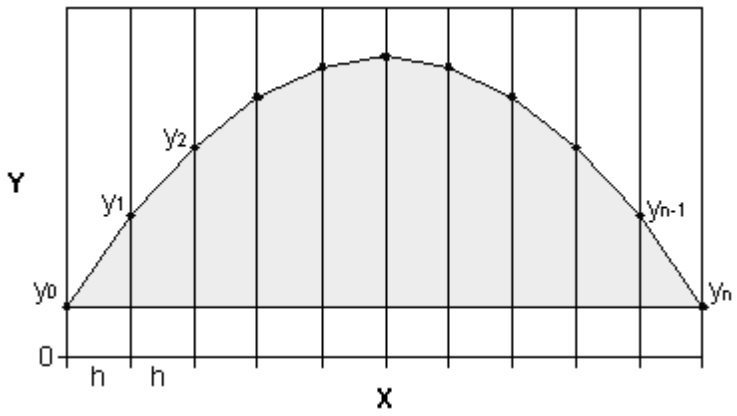


Figure 2.1.5 Visceral warming pattern measured using *Archtag* analysis software. t_{max} – Time to reach maximum temperature, MH – Maximum heat increase, FM – Feed Measure, area under the visceral warming pattern, FD – Feed Duration (min).



yj= visceral temperatures (°C) at each sampled time step

yb = the temperature at the beginning of the feeding event (i.e. basal temperature for this experiment)

h= sampling interval

Figure 2.1.6 An approximated visceral warming curve from connecting adjacent points by a straight line. The shaded area was calculated by trapezoidal integration (Gunn et al., 2002).

Data and tag scrutiny

Recordings were rejected due to:

1. uncertainty about which SBT ate particular rations due to competitive feeding behaviour and being unable to exactly identify the fish that consumed the ration,
2. uncertainty due to unfavourable weather conditions about which SBT consumed baitfish. This was either due to rough weather obscuring vision of the identifying tag or the fish being fed and no data recordings being made, and
3. archival tag data showing that the SBT had consumed a meal when it had not been offered a meal. This is likely to have arisen when live baitfish entered the pontoon, when local tour operators fed the SBT, and when a feeding event had not been recorded.

Some tag data were not downloaded as a result of the archival tag itself being lost due to mortality or poaching. Vemco tags failed in Trial 1 leading to erratic temperature recordings which possibly came about due to the ageing loggers and power supply. Loggers with new batteries were used to monitor water temperature (T_w). To maintain consistency in Trial outcomes, data derived from Vemco tags was rejected, and DST-Centi tags (Star-Oddi, Iceland) data was used for this research.

Analysis of baitfish composition

In many of the Trials, it was necessary to assess the nutritional composition of baitfish to determine the rate and duration of visceral warming in SBT and the time taken for visceral warming to reach t_{max} in response to the baitfish ration and energy consumed. The following method was used to determine proximate composition of baitfish.

Preparing baitfish for proximate analysis required a 1 to 2 kg baitfish sample that was randomly selected to be macerated in a commercial meat mincer three times until homogenous. A 700 g sub - sample was placed in a plastic food grade takeaway container and frozen at -18^{o}C until a suitable quantity of samples was accumulated to send for analysis by direct courier delivery method to Weston Food Laboratories, Sydney for analysis according to the methods outlined in Table 2.1.1.

Table 2.1.1 Analytical methods to test baitfish nutritional parameters.

Parameter	***Analytical method***
Protein (kJeldahl)	AOAC 988.05
Lipid (soxhlet)	AOAC 960.39
Moisture	AOAC 950.46
Energy	Calculated (Lazo and Davis, 2000)

AOAC – Association of Analytical Communities

Determining nutritional composition of baitfish mixes

Ellis and Rough (2005) assessed the quality and nutritional profiles of baitfish used in the SBT ranching industry, and provide a guide on the nutritional profiles of baitfish commonly used for feed in SBT ranching operations. The results of the research underpinning the baitfish nutritional profiles were incorporated into an Excel software program *FORMU-BAIT*© that optimises nutrient inputs (van Barneveld and Ellis, 2007). *FORMU-BAIT*© was used to consider the nutritional composition of various species of baitfish, the results of which were used to formulate a mix of baitfish meeting known amino acid and fatty acid profiles of SBT (van Barneveld and Ellis, 2007).

2.2 Trial 1 – The visceral warming response in southern bluefin tuna (*Thunnus maccoyii*) to a single meal with an emphasis on volume of feed ingested, dietary energy and baitfish size

This Trial was conducted at the end of the 2003/2004 ranching season at the southern end of Boston Island in a sheltered bay (Rotten Bay). Rotten Bay was the preferred location for the research due to the protection it offered from prevailing weather conditions.

SBT were caught at -33^{o} 36’ and 132^{o} 25’ and towed to Port Lincoln arriving on 10 March 2004.

Initially 40 SBT were inserted with archival tags comprising n=20 DST-Centi tags (Star-Oddi, Iceland) and n=20 Minilog 12 bit tags (Vemco, Canada). The tags were inserted into SBT on 8 April 2004 and these SBT were placed into a 40 m diameter ranching pontoon comprising a 450 mm diameter polyethylene buoyancy ring filled with two pack foam (DMS, Australia) with a nylon 415 ply knotless 150 mm stretched net (Badinotti, Italy) and 10 m net walls as part of an earlier scoping trial. The pontoon volume was 12568 m^3. The pontoon was subsequently towed by the F.V. *Lucky-S* on 15 August 2004 from the tuna farming offshore zone to Rotten Bay on the southern side of Boston Island (Figure 2.2.1). The ranching pontoon was moored on arrival and the SBT were fed for a period of one week with fresh local sardines (*S. sagax*) coated with a standard vitamin premix to assist recovery from any stress associated with the transfer and tow, and to prepare them for handling.

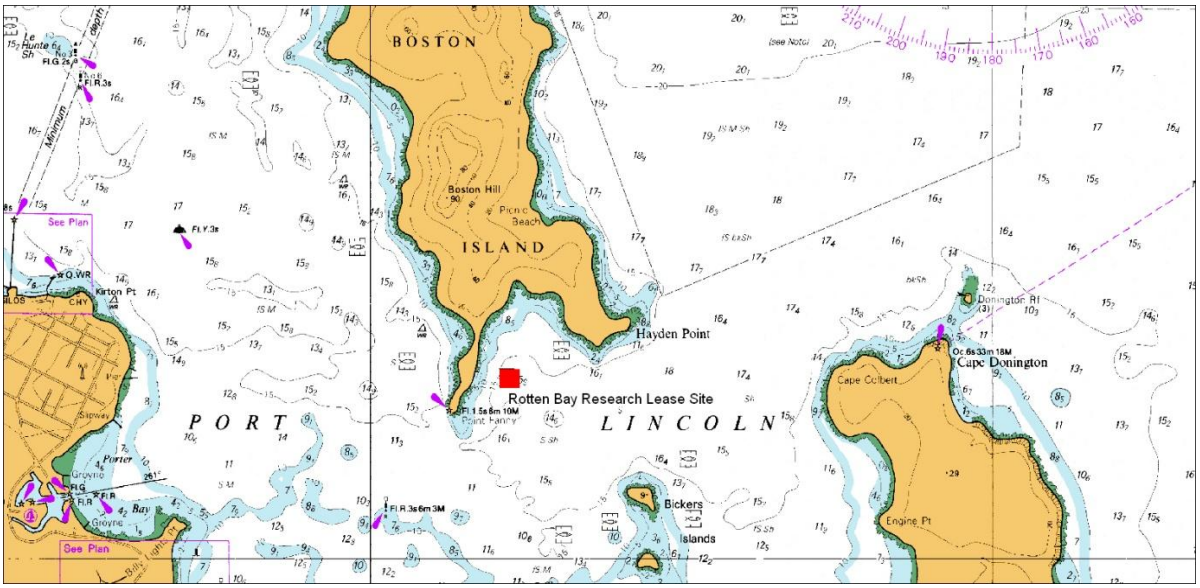


Figure 2.2.1 Southern bluefin tuna 5ha research lease site shown in red located in the sheltered waters of Rotten Bay on the southern end of Boston Island, Port Lincoln.

A baited barbless hook and handline was used to catch SBT from the 40 m diameter ranching pontoon. Once caught the SBT were pulled onto a vinyl lined mattress positioned between the 40 m diameter pontoon (DMS, Australia) and 12 m diameter pontoon (Polar Cirkel, Norway) with nylon 415 ply knotless 150 mm stretched net with 8 m deep net walls attached (Badinotti, Italy). The volume of the 12 m diameter pontoon was 905 m^3.

Conventional dart tags (Hallprint, Australia) were inserted on the left side, right side or both sides of the second dorsal fin or not at all so each of four SBT could be recognised visually during feeding. Following tagging the SBT were placed into a 12 m diameter pontoon, and the same method was repeated to create four replicate pontoons stocked with four SBT per pontoon.

The trial started on 9 September 2004. The feeding regime was: 0900 h on Day 1, 1500 h on Day 2 and no feeding on Day 3. The food deprivation on Day 3 aimed to allow visceral temperature (T_{vis}) to return to T_b thus producing a discrete T_{vis} pattern based on a single meal (Gunn et al., 2002). The baitfish used in this Trial were sourced from the United States of America (*S. sagax*), Australia (*S. sagax*), and Morocco (*Sardina pilchardus).*

Prior to each feeding event a 20 kg block of baitfish was thawed to T_w and individual baitfish of a common length ±1 cm were selected for feeding. A 2 kg representative sample was taken for proximate testing. Each piece of bait was weighed and a mean value determined from all weights recorded. At each feeding event, SBT were offered a single whole baitfish and the SBT that ate the baitfish was recorded according to the dart tag pattern. Once a SBT in the pontoon had consumed approximately 1 kg of baitfish, feeding ceased and the process was repeated for the remaining pontoons. Intake (g) was determined by multiplying the mean value of the baitfish from the 2 kg sample by the number of pieces of baitfish consumed. Pontoon order and baitfish type to be fed were randomly selected.

The Trial ran over 24 days between 9 September and 3 October 2004 and was completed once the SBT in each of the four stocked 12 m diameter pontoons had been fed four times with each of the four baitfish types reflecting different energy content (resulting in 16 feeding events per pontoon).

T_w was recorded using a calibrated Vemco temperature data logger (Vemco, Canada) suspended from the pontoon at a depth of 5 m. This was based on research showing the water column was well mixed both vertically and horizontally (Petrusevics, 1993; Hone et al., 1996).

At the completion of the Trial the SBT were harvested, and the archival tags Star – Oddi DST Centi (Star-Oddi, Iceland) Minilog 12 bit tags (Vemco, Canada) were downloaded and analysed. A total of 130 data recordings were available from a potential total of 256 feeding

due to battery failure and of these 76 recordings were validated for use as defined in the tag scrutiny section in the general research methods.

Statistical analysis

The objectives of this Trial were to determine the relationship between indicators of visceral warming and feed intake and dietary energy. The following measures were used to assess visceral warming patterns (i.e. dependent variables):

a) Feed Measure (FM) – Feed Measure of area under heating curve
b) Feed Duration (FD) – time taken from the beginning to the end of the feed measurement (in minutes)
c) Time max (t_{max}) – time taken to reach maximum visceral warming

The following were used as predictors following Gunn et al., (2002):

a) Dietary energy - total energy consumption (kJ). Dietary energy was used as a predictor for FI and FD
b) Feed intake (FI) was used as a predictor for t_{max}. Feed Intake was assessed as a percentage of harvest body weight, calculated as: feed weight (g) / harvest weight (g), multiplied by a factor of 100 to simplify the metric
c) Feed type was also included in these analyses to assess for baitfish differences and homogeneity of slopes between the various parameters

The relationships were assessed using univariate General Linear Modelling (GLM) procedures. ANOVA was used to compare the FM of the different baitfish. The combined relationship of feed type and dietary energy on FM was assessed using ANCOVA.

Similarly, the relationship between feed type and dietary energy on feed duration was assessed with ANCOVA. Finally, the effects of feed intake and feed type on t_{max} were evaluated with ANCOVA. If required, the Least Square Difference (LSD) post-hoc test was applied at a significance level of α=0.05, to determine differences between the means of categorical explanatory variables.

The SPSS statistical package version 17 (©2008, SPSS Inc.) was used to analyse all data.

Charts were prepared using Microsoft® Office Excel® 2007 (Microsoft Office Professional 2007©2006 Microsoft Corporation).

2.3 Trial 2 – The visceral warming response to one, two or three feeds in southern bluefin tuna (*Thunnus maccoyii*) with an emphasis on weight of feed ingested and dietary energy at two different water temperatures

This Trial was designed to build on the results of Trial 1 by considering the influence of different water temperatures and feeding events on visceral warming patterns. The vigorous feeding behaviour of new SBT introduced into pontoons tend to 'smash' baitfish making it extremely difficult to identify which SBT consumed pieces of baitfish. As a consequence, SBT used in this Trial were held through winter and in to the following summer to limit the influence of feeding behaviour on physiology measurements.

Two year old SBT were captured on 26 February 2006 at -33^{o} 50' and 132^{o} 25' and transported to a site near Port Lincoln on 14 March 2006. DST-Centi archival tags (Star-Oddi, Iceland) were inserted into 20 SBT on 30 April 2006 and SBT transferred into a 12 m diameter pontoon (Plastic Fabrications, Tasmania) made of nylon 415 ply knotless 150 mm stretched net with 8 m deep net walls attached (Badinotti, Italy). The volume of the pontoon was 905 m^3.

The archival tags were set to commence logging data from 26 June 2006 so that calibration data and feeding behaviour information could be taken at different water temperatures (T_w):

- Period 1 - November 2006 ≈ 17^{o}C
- Period 2 - February 2007 ≈ 22^{o}C

A baited barbless hook size 8/0 (Diamond – Yamamoto, Japan) and handline was used to catch 12 SBT from the 12 m diameter pontoon (Plastic Fabrications, Tasmania) on 10 October 2006.

SBT length was measured using a calibrated ruler and weight was estimated based on applying a base conversion condition index from a known length weight relationship (Polacheck et al., 2004) for SBT with the following equation:

Weight (kg) = Length $(m)^3$ * CI

Once caught the SBT were pulled onto a vinyl lined mattress positioned between the 12 m diameter pontoon (Plastic Fabrications, Tasmania) and the waiting 12 m diameter pontoon (Plastic Fabrications, Tasmania). Conventional dart tags (Hallprint, Australia) were inserted on the left side, right side or both sides of the second dorsal fin or not at all so that each of four SBT could be recognised visually during feeding.

Following tagging, the SBT were placed into a 12 m diameter pontoon (Plastic Fabrications, Tasmania) made of nylon 415 ply knotless 150 mm stretched net with 8 m deep net walls attached (Badinotti, Italy). The volume of the pontoon was 905 m^3. The same method was repeated to create three replicate pontoons stocked with four SBT per pontoon.

The SBT were fed using the same regime during Periods of two different water temperatures (T_w). The feeding regime involved feeding for six days with no feeding on the seventh day before starting the next feeding regime. The feed times were at 9:00 h, 12:00 h and 15:00 h for three feeds per day, at 9:00 h and 15:00 h for two feeds per day and at 12:00 h for a single feed per day. The Trial was completed after three weeks when the SBT in all three pontoons had been fed once per day for one week, twice per day for one week, and three times per day for one week.

The baitfish used in this Trial was Australian sardines (*S. sagax*). Prior to each feeding event in the first stage of this Trial, a 20 kg block of baitfish was thawed to ambient T_w and individual baitfish of a common length ±1 cm were selected for feeding. A 2 kg representative sample was taken for proximate testing. Each piece of bait was weighed and a mean value determined from all weights recorded. At each feeding event, SBT were offered a single whole baitfish and the SBT that ate the baitfish was identified according to the dart tag pattern. At each feeding event, SBT identified by the dart tag pattern were offered single whole pieces of baitfish until satiation (which was determined when two pieces of bait fell out of sight).

Each piece of baitfish consumed by an individual SBT was recorded according to the dart tag pattern. Following cessation of feeding the process was repeated for the remaining pontoons.

Intake was determined by multiplying the mean value of the baitfish from the 2 kg sample by the number of pieces of baitfish consumed. Pontoon order and feed frequency for the Trial were randomly determined.

At the second stage of the Trial, fresh caught Australian sardines (*S. sagax*) from Spencer Gulf that had been frozen in 1 kg flat packs for use in the Trial were selected based on a mean weight and length. A 2 kg representative sample was taken for proximate testing. The mean baitfish weight was determined by dividing the total weight of the 1 kg flat pack by the number of baitfish contained in the flat pack.

At each feeding event, baitfish was thawed to ambient T_w and SBT were offered a single whole baitfish. The SBT that ate the baitfish was identified according to the dart tag pattern. At each feeding event, SBT identified by the dart tag pattern were offered single whole pieces of baitfish until satiation. Following the cessation of feeding the process was repeated for the remaining pontoons. Intake was determined by multiplying the mean value of the baitfish from the flat pack by the number of pieces of baitfish consumed. Pontoon order and feed frequency for the Trial were randomly determined.

T_w was recorded using a calibrated Vemco temperature data logger (Vemco, Canada) suspended on the pontoon at a depth of 5 m.

At the completion of the Trial the remaining 7 SBT were harvested, lengths and weights recorded, the archival tags were retrieved, downloaded and analysed.

Statistical Analysis

The objective of this Trial was to determine the relationship between indicators of visceral warming and feed frequency, feed intake and dietary energy. The following measures were used to assess visceral warming (i.e., dependent variables):

a) Feed Measure (FM) – Feed Measure of area under the heating curve

b) Time max (t_{max}) – time taken to reach maximum visceral warming

The following were used as predictors:

a) Feed frequency – the number of feeds ranged between 1 and 3 per day

b) Dietary energy – total energy consumption (kJ). Dietary energy was used as a predictor for FI

c) Feed intake was used as a predictor for t_{max}. Feed Intake (FI) was assessed as a percentage of harvest body weight, calculated as: feed weight (g) / harvest weight (g), multiplied by a factor of 100 to simplify the metric

The relationships were assessed using univariate General Linear Modelling (GLM) procedures. Differences in feed measure or t_{max} as a function of feed frequency were assessed using ANOVA. The effects of feed frequency and dietary energy on feed measure (FM) were assessed using ANCOVA. Similarly, the effects of feed frequency and feed intake on t_{max} were evaluated using ANCOVA. Analyses were conducted separately on data obtained through Trials conducted in November 2006 ≈ 17^{o}C and February 2007 ≈ 22^{o}C. Potential differences in the relationship between dietary energy and FM according to the time of the Trial (i.e. Period 1 - November 2006 ≈ 17^{o}C vs. Period 2 - February 2007 ≈ 22^{o}C) were investigated using ANCOVA. The Least Square Difference (LSD) post-hoc test was applied where applicable at a significance level of α=0.05, to determine differences between the means of the categorical explanatory variable (i.e. feed frequencies).

The SPSS statistical package version 17 (©2008, SPSS Inc.) was used to analyse all data. Charts were prepared using Microsoft® Office Excel® 2007 (Microsoft Office Professional 2007©2006 Microsoft Corporation).

Trial 1 and 2 combined - to determine the relationship between basal and maximum visceral temperatures and water temperature

A challenge arising during Trial 2 was how to determine basal visceral temperature in SBT that had consumed either two or three meals.

To measure and interpret visceral warming patterns, basal visceral temperature and maximum visceral temperature data from Trials 1 and 2 were combined to enable consideration of the relationships between basal visceral temperature (T_b) and maximum visceral temperatures (T_{max}) and ambient T_w.

Statistical Analysis

Data from Trials 1 and 2 were analysed using univariate General Linear Modelling (GLM). Results were interpreted using the SPSS statistical package version 17 (©2008, SPSS Inc.) Charts were prepared using Microsoft® Office Excel® 2007 (Microsoft Office Professional 2007©2006 Microsoft Corporation).

2.4 Trial 3 – The measurement of temperature in red muscle, white muscle and the visceral cavity of slaughtered southern bluefin tuna (*Thunnus maccoyii*) in response to three feeding regimes and ambient water temperature

The objective of this Trial was to investigate whether feeding practices and water temperature (T_w) influence red muscle temperature (T_{rm}), white muscle temperature (T_{wm}) and visceral temperature (T_{vis}) in SBT.

SBT were caught at -33^o 19' and 131^o 48' on the 17 January 2005. The captured SBT were raised under commercial conditions in different ranching pontoons. For the purpose of this Trial the captured SBT were subjected to three feeding regimes before being harvested. The three feeding regimes were: feeding during the afternoon on the day before harvest (12 h); feeding during the morning on the day before harvest (24 h); and no feeding during the day before harvest (48 h).

SBT were harvested on 25, 27 and 29 April, 10 May, 6 and 27 June and 1 July for the purpose of this trial.

A Gemini temperature data logger Model TV-4050 (Hastings Data Loggers, Sydney, Australia) connected to a 5 m cable with a 100 mm temperature probe was used to monitor temperature in three body locations of individual SBT following harvest. Prior to harvest,

the temperature probe was placed in the sea at a depth of 5 m for a period of ten minutes to record ambient T_w.

Ten SBT were randomly selected during the harvest for sampling. Once harvested and slaughtered the temperature probe was placed through the 2 cm long x 2 cm deep bleed cut made using a small triangular knife through the dorsal ridge of the cavity that the pectoral fins fold back into during periods of high propulsion swimming, and into the red muscle and allowed to stabilise for a period of two minutes. The probe was then removed, allowed to cool and sterilised using ethanol before placing it into the white muscle by inserting the needle in a dorsal ventral direction at the base of the third fin ray of the first dorsal fin and allowed to stabilise for two minutes. The probe was then removed and allowed to cool and sterilised using ethanol and placed into the visceral cavity through the incision that was made through the belly wall just in front of the vent where the intestine was pulled through the opening as part of the harvest process. This process was repeated for each harvested SBT. The total time taken to sample temperatures at the three body sites for each SBT was no more than seven minutes. Weight and length of each SBT were also recorded.

Statistical Analysis

Dependent variables consisted of temperatures recorded from the red and white muscle, and visceral cavity. The predictors consisted of:

a) Feeding regimes – three regimes were used: no feed for 48 hours, no feed for 24 hours, and no feed for 12 hours

b) Ambient T_w– this was included as it is a distinct predictor of visceral warming

The relationships were assessed using univariate General Linear Modelling (GLM). Specifically, separate ANCOVAs were conducted to predict each body temperature from the treatment group and the sea water temperature. Where relevant, the Least Square Difference (LSD) post-hoc test was applied at a significance level of α=0.05, to identify significant differences between levels of the categorical explanatory variable (i.e. feeding practices).

The SPSS statistical package version 17 (©2008, SPSS Inc.) was used to analyse data. Charts

were prepared using Microsoft® Office Excel® 2007 (Microsoft Office Professional 2007©2006 Microsoft Corporation).

2.5 Trial 4 – The measurement of maximum and basal visceral temperature in commercially cultured southern bluefin tuna (*Thunnus maccoyii*) in response to commercial feeding practices at ambient water temperatures

The objective of this Trial was to investigate the relationship between T_b and T_{max} in relation to T_w in commercial cultured SBT. Commercial data and experimental Trial data were compared to determine differences in T_b or T_{max} and whether tagging has an influence on physiology responses in SBT.

Archival tags (Wildlife Computers, USA) were inserted into free roaming SBT by CSIRO (using methods consistent with those described in Section 2.1) in the Great Australian Bight. Tagging was conducted between 2004 and 2008 as part of a Fisheries Research and Development Corporation (FRDC) funded research project: *Spatial interactions among juvenile southern bluefin tuna at the global scale: a large scale archival tag experiment*. The tags track spatial movements of SBT by relating the initial release point with a nuclear clock recording Greenwich Mean Time in response to light level recorded on the external sensor and depth sensor measurements. The tags were programmed to record data either every second or every 30 seconds.

CSIRO provided the data from twelve tagged SBT that were caught by purse seine fishing vessels and ranched from summer through to winter/spring. Data provided by CSIRO was T_{vis} and T_w data from the point of capture to harvesting in the context that the data had not been used for any other research purpose and was irrelevant to the physiology of free roaming SBT. The data was provided in an Excel CSV format (Microsoft® Office Excel® 2007 Part of Microsoft Office Professional 2007©2006 Microsoft Corporation), and required standardisation for consistency with the programming of archival tags used in this research. Standardisation was accomplished by filtering the data and setting up a pivot table to screen data for use in the analysis.

Three temperature measurements were obtained from the CSIRO data: T_w, T_b, and T_{max}.

Data from Trials 1, 2 and 3 were also included in this section to enable comparison:

- the T_w to T_b relationship - data obtained from Trials 1 and 2
- the T_w to T_{wm} relationship - data obtained from Trial 3
- the T_w to T_{rm} relationship - data obtained from Trial 3

Statistical Analysis

Data were assessed using univariate General Linear Modelling (GLM). ANOVA was used to compare the T_b and T_w between data obtained from Trials 1 and 2, and Trial 3. Linear regression was used to determine whether T_b and T_{max} could be predicted from T_w in the CSIRO data. Separate linear regressions were conducted with T_b or T_{max} as the dependent variable, and T_w as the predictor.

ANCOVA was used to investigate any differences in relationship between T_b and T_w and between T_{max} and T_w. In this Trial, the temperature value was the dependent variable, T_w was entered as a continuous predictor (covariate) and the temperature type (i.e. T_b or T_{max}) was dummy coded. A manually specified interaction between the dummy coded temperature type and T_w was used to test whether the relationship between body and T_w differed according to temperature recording types.

Dummy coding and ANCOVA with interaction was used to evaluate whether there were structural breaks in the T_w to T_b or T_{max} relationships (Chow test for equality of coefficients). The dummy variable was also used to assess the difference in intercepts for the two temperature groups, and any difference in slopes. The same method (i.e. ANCOVA analysis with dummy coding and interaction) was used to determine whether the T_b to T_w relationship differed between the data obtained from Trials 1 and 2, and Trial 3. Where applicable, the Least Square Difference (LSD) post-hoc test was applied, at a significance level of α=0.05, to determine differences between the categorical explanatory variables.

The SPSS statistical package version 17 (©2008, SPSS Inc.) was used to analyse data. Charts were prepared using Microsoft® Office Excel® 2007 (Microsoft Office Professional 2007©2006 Microsoft Corporation).

2.6 Trial 5 – Feed intake, FCR, growth and proximate composition of southern bluefin tuna (*Thunnus maccoyii*) fed four diets in three periods over an 18 week period: *Can feed intake from visceral warming patterns be predicted?*

The objectives of this Trial were to utilise *FORMU-BAIT*© feed optimisation software (van Barneveld and Ellis, 2007; van Barneveld et al., 2009) to formulate combinations of baitfish diets varying in protein and lipid content to optimise SBT growth and feed intake over the ranching season. The Trial investigated the response of treatments (feeding regimes) on intake, growth, Food Conversion Ratio (FCR), Specific Growth Rate (SGR) and SBT flesh proximate composition. The objectives of this Trial also involved investigation of the relationship between visceral warming patterns and dietary energy according to four feeding regimes. Analyses were conducted within each Period and between Periods to assess any differential effects of the treatments. General Linear Models developed from Trials 1 and 2 were applied to assess whether feed intake could be predicted based on visceral warming patterns.

SBT were caught at - 33° 27' and 132° 04' between 19 February and 3 March 2005 by a purse-seine vessel and transferred into a pontoon which was towed to the tuna farming offshore zone near Port Lincoln. On arrival (28 March 2005), the SBT were transferred from the tow pontoons into static commercial ranching pontoons.

The SBT ranching pontoon consisted of a 50 m by 450 mm diameter polyethylene buoyancy ring filled with two pack foam (DMS, Australia) comprising nylon 415 ply knotless 150 mm stretched net with 10 m deep walls attached (Badinotti, Italy). The volume of the pontoon was 19638 m^3.

An initial sample of ten SBT was taken from the 50 m diameter pontoon on 5 April 2005 for SBT flesh proximate analysis to determine the mean baseline composition.

Between 5 and 10 April 2005, a total of 879 SBT were caught using a baited hook and handline and hauled into a waiting aluminium cradle on a harvest pontoon positioned on the edge of the 50 m pontoon (Figure 2.6.1). SBT length was measured and, when ocean conditions permitted, weights were recorded.

SBT were tagged with a conventional dart tag (Hallprint, Australia) and placed into one of four 32 m diameter research pontoons until approximately 220 SBT had been transferred into each pontoon. Two pontoons consisted of a single 32 m diameter x 450 mm polyethylene buoyancy ring (DMS, Australia) and two pontoons were double polyethylene collar (315 mm outside and 450 mm inside) (Plastic Fabrications, Tasmania). All four pontoons were fitted with nylon 415 ply knotless 150 mm stretched nets with 9 m net walls (Badinotti, Italy). The volume of the each pontoon was 7240 m^3.

Figure 2.6.1 Capturing SBT using a baited barbless hook and handline from a 50 m diameter commercial ranching pontoon before transferring into a handling cradle to measure length and weight, tagging with conventional numbered dart tag, and transferring into a 32 m diameter research pontoon.

Of the 879 tagged SBT, 108 were accurately weighed and measured due to prevailing weather conditions. To establish a Fulton Type base level condition index factor (Jones et al., 1999), the following equation was applied:

Weight (kg) = Length $(m)^3$ * CI

The resultant condition index of 18.21 was then used to calculate the weight of those SBT that had not been measured directly based on their measured length.

Once transferred into the research pontoons, the SBT were fed the same diet consisting mainly of local Australian sardines (*S. sagax)* for eight days until 18 April 2005 when the experiment commenced and DST-Centi archival tags (Star-Oddi, Iceland) were inserted into ten SBT from each pontoon.

Treatments were fed to the SBT in each of the pontoons during three Periods:

- Period 1 – 18 April to 30 May 2005
- Period 2 – 1 June to 11 July 2005
- Period 3 – 12 July to 22 August 2005

SBT were harvested from each pontoon at the end of each six week Period (30 May 2005, 11 July, and 22 August 2005). SBT in each pontoon were shovel fed specific baitfish ratios two times per day to satiation for each six week Period when weather conditions permitted. Treatment feeding regimes were randomly allocated to the pontoons.

For the first harvest (May), ten SBT were sampled from each pontoon. A further 20 SBT were to be caught by baited barbless hook and handline from each pontoon for length and weight measuring, however the SBT would not accept the baited hook. The first sampling was therefore restricted to ten SBT per pontoon. For the two remaining harvests (July 11 and 22 August), 30 SBT were harvested from each pontoon, from which ten SBT were destructively sampled for flesh proximate composition.

All destructively sampled SBT were gilled and gutted (GG) and an identifier tag placed on the tail. All weights and lengths from individual SBT were recorded during processing. As it was impossible to record whole weights of each SBT due to sea conditions, the whole weight of individual SBT was calculated using an industry standard of 13% harvest weight loss (resulting from the removal of gills, viscera and blood).

One entire fillet from each destructively sampled SBT was minced until homogenous and sent for proximate analysis (Weston food laboratories, Sydney Australia). In addition, a 2 kg sample from every baitfish type used was randomly sampled at every feeding event and stored in a plastic bag with a unique identifier in commercial freezers at -20^{o}C.

These samples were pooled each week, based on their type, fed through a commercial meat mincer until homogenous and sent for proximate analysis.

Selection of baitfish and diet formulation

Proximate analysis was performed on 22 shipments of various baitfish species held in freezers by a commercial SBT ranching company. This analysis involved mincing a 20 kg block randomly selected from a given shipment and removal of a 700 g sub-sample for analysis.

Proximate results of this analysis were entered into *FORMU-BAIT*© (van Barneveld and Ellis, 2007) and used to identify combinations of baitfish for supply of high protein (≈19.5%) and low lipid (≈4%), medium protein (≈18.5%) and medium lipid (≈7.5%), and low protein (≈17.5%) and high lipid (≈10%). Baitfish subsequently selected for the Trial were sardines (*S. sagax)* sourced from the United States of America, red bait (*Emmelichthys nitidus nitidus)* sourced from Tasmania, sardines (*S. sagax)* sourced locally from Port Lincoln, and sardines (*S. sagax*) sourced from the East Coast of Australia (Table 2.6.1). The *FORMU-BAIT*© ratios used to develop baitfish combinations for feed specifications are shown in Table 2.6.2

Feeding strategies

Four treatments (feeding regimes) were developed to examine the response of SBT to varying nutrient supply delivered in three time Periods during a growing season. The four regimes involved consistent supply of medium protein and medium lipid (MP/ML) for the duration of the Trial, supply of locally caught sardines only, and supply of either low protein and high lipid progressing to high protein and low lipid or vice versa (Table 2.6.3).

Table 2.6.1 Nutrient proximate analysis and energy of baitfish used in Trial 5.

Parameter	Protein %	Moisture %	Lipid %	Gross energy MJ/kg
Sardinops sagax (US)	16.35	63.80	14.87	8.63
Emmelichthys nitidus nitidus	19.80	72.30	2.50	4.29
Sardinops sagax (EC)	20.35	73.80	2.00	4.96
Sardinops sagax (PL)	20.40	70.80	4.30	5.21

Note – All proximate results provided by laboratory. Sardinops sagax (US) sourced from the United States of America, Sardinops sagax (EC) sourced from the east coast of Australia and Sardinops sagax (PL) sourced from Port Lincoln, Australia.

Table 2.6.2 Research Trial nutrient specifications and formulated baitfish combinations to meet the nutrient specifications as determined by *FORMU-BAIT* ©.

Feed Specifications	Protein %	Lipid %	*Sardinops sagax* (US) %	*Emmelichthys nitidus nitidus* %	*Sardinops sagax* (EC, PL) %
High protein/Low lipid	19.50	4.0	15	30	55
Medium protein/Medium lipid	18.50	7.3	40	35	25
Low protein/High lipid	17.50	10.5	65	35	
Local sardines	20.4	3.6-4.3			100

Note – Table shows protein and lipid values for feeding regime, and baitfish % required to meet feeding specification of total.

Table 2.6.3 Experimental treatments (feeding regimes) used in Trial 5.

Treatment (Pontoon)	Period 1 0-6 wk	Period 2 7-12 wk	Period 3 13-18 wk
1 (10)	MP/ML	MP/ML	MP/ML
2 (11)	LP/HL	MP/ML	HP/LL
3 (12)	HP/LL	MP/ML	LP/HL
4 (13)	Local	Local	Local

Note - MP – medium protein; ML – medium lipid; HP – high protein; HL – high lipid; LP – low protein; LL – low lipid; Local – locally caught sardines

Statistical Analysis

Analyses were performed on the following indexes of SBT performance and quality for each Trial Period and between Trial Periods to assess the influence of different feed regimes over time:

- Condition Index – calculated as whole Wt (kg) / $L(m)^3$
- Growth
    - Weight Increase: whole final weight - initial calculated weight (kg)
    - Length increase: final length - initial length (cm)
- Food Conversion Ratio (FCR) = total food consumed Wt(kg) / (Wt Final (kg) - Wt Initial (kg)
- Food Intake, calculated as food fed to pontoon: weight consumed (g) / body weight (g) * 100, and consumption per SBT per day
- Specific Growth Rate (SGR), specific growth rate to provide a measure of daily weight gain: calculated as SGR = [ln(Wt final) - ln(Wt initial)]/(Time final – Time initial) * 100

*Note: abbreviations used in formula / = divided, * = multiplied, and - = subtract*

In addition, carcass quality as determined by protein, lipid and moisture content was analysed (Weston Food Laboratories).

Relationships were assessed through univariate General Linear Modelling (GLM). Separate ANOVAs were conducted to evaluate the implications of different feeding regimes on SBT quality and performance. Where relevant, the Least Square Difference (LSD) post-hoc test was applied at a significance level of α=0.05 to determine mean differences, though results were considered in the context of the number of comparisons and the concomitant Type I error rate. That is, the overall trends were considered rather than specific significance test results.

Measurement of visceral warming

Visceral warming patterns were analysed for all SBT involved in the different feeding regimes as follows: at the beginning of week four for six days during the first Period; at week 10 for five days in the second Period; and at week 16 for six days in the third Period.

This analysis method enabled comparison of visceral warming associated with each feeding regime for every Period and between treatment Periods. Analyses were conducted at week 4 of each Period as this proved to be the most consistent feed week (i.e. there were no disruptions to feeding).

T_b was determined by applying the GLM that was developed from Trial 2 ($y=0.7723x+7.4119$; $R^2=0.9$). In order to assess whether FM was a good predictor of feed intake, models developed during Trials 1 and 2 were used to model the data obtained during this Trial.

T_w was recorded using a calibrated Vemco temperature data logger (Vemco, Canada) suspended on the pontoon at a depth of 5 m.

Statistical Analysis

The SPSS statistical package version 17 (©2008, SPSS Inc.) was used to analyse data obtained in this Trial. Charts were prepared using Microsoft® Office Excel® 2007 (Microsoft Office Professional 2007©2006 Microsoft Corporation).

2.7 Trial 6 – Measurement of visceral warming patterns in commercially grown southern bluefin tuna (*Thunnus maccoyii*) in response to two feeding regimes

The objective of this Trial was to investigate the influence of feeding regimes on visceral warming of SBT grown commercially by two ranching companies.

Site 1: Sekol Farmed Tuna

SBT were caught at -33° 29' and 132° 36' between 8 and 14 March 2007 by a purse-seine vessel and transferred into a tow pontoon. The pontoon was towed to the tuna farming offshore zone near Port Lincoln, arriving on 31 March 2007. SBT were transferred from the tow pontoon into a static ranching pontoon on 1 April 2007. The ranching pontoon comprised 40 m by 450 mm diameter polyethylene buoyancy ring filled with two pack foam (DMS, Australia) with nylon 415 ply knotless 150 mm stretched net with 10 m deep walls attached. The volume of the pontoon was 12568 m^3.

Fifteen SBT were inserted with DST-Centi archival tags (Star-Oddi, Iceland) on 12 April 2007. A calibrated Vemco data logger (Vemco, Canada) was placed on the net of the pontoon at a depth of 5 m to record T_w. SBT were fed up to twice per day to satiation using a range of baitfish.

Site 2: Kistuna

SBT were caught at -33° 03' and 131° 01' on 13 February 2007 by a purse-seine vessel and transferred into a tow pontoon. The pontoon was towed to the tuna farming offshore zone near Port Lincoln, arriving on 6 March 2007. SBT were transferred on 7 March into static ranching pontoons comprising a 40 m by 450 mm diameter polyethylene buoyancy ring filled with two pack foam (DMS, Australia) with nylon 415 ply knotless 150 mm stretched net with 10 m deep walls attached. The volume of the pontoon was 12568 m^3.

Fifteen SBT were inserted with DST-Centi archival tags (Star-Oddi, Iceland) on 5 April 2007, and a Vemco data logger (Vemco, Canada) was placed on the net of the pontoon at a depth of 5 m to record T_w. SBT were fed up to six times per day with predominantly locally-caught sardines (*S. sagax*).

T_w was recorded using a calibrated Vemco temperature data logger (Vemco, Canada) suspended on the pontoon at a depth of 5 m.

Statistical Analysis

Visceral warming patterns were analysed at three different T_w. as follows:

- Period 1 - 19 – 24 April (mean T_w 18.8 ^oC)
- Period 2 - 20 – 25 May (mean T_w 17.0 ^oC)
- Period 3 - 21 – 26 June (mean T_w 15.1 ^oC)

This method enabled comparison of visceral warming with regard to the influence of water temperature. T_b was determined by applying the GLM from Trial 2 ($y=0.7723x+7.4119$; $R^2=0.9$).

Each company recorded feeding information including the time and date the SBT were fed, type of feed, feeding method and volume. The SBT were harvested, archival tags retrieved and data was analysed using CSIRO *Archtag* software.

Feed measure (FM) was used as the measure of visceral warming, and the following predictors of FM were investigated:

- Company – *Sekol Farmed Tuna* vs. *Kistuna*
- Time period
- Number of times SBT were offered food
- Number of times SBT consumed food in relation to how many meals were offered

Relationships were assessed using univariate General Linear Modelling (GLM). ANOVA was used to compare the influence of predictors on the FM. Linear regressions were used to determine whether FM could be significantly predicted by the number of feeding events or meals consumed. ANCOVAs were used to evaluate whether the feeding regime (offered or consumed) to FM relationships differed as a function of the company. Company was the factor, number of feedings was the continuous covariate, and the FM was the dependent variable. ANCOVAs were used to investigate whether the differences between meals

offered and consumed were related to FM or company. Where relevant, the Least Square Difference (LSD) post-hoc test was applied at a significance level of α=0.05, to determine differences between the means of the categorical explanatory variables (i.e. time period).

The SPSS statistical package version 17 (©2008, SPSS Inc.) was used to analyse all data. Charts were prepared using Microsoft® Office Excel® 2007 (Microsoft Office Professional 2007©2006 Microsoft Corporation).

Chapter 3 - Results

This chapter presents the results of the six Trials that form this study. Data showing influences on visceral warming patterns are presented and the influence of feeding on regional endothermy in red and white muscle tissue is examined. Nutrition data are evaluated and results of investigations into relationships between feeding regimes, visceral warming and performance measures are detailed.

3.1 Trial 1 – The visceral warming response in southern bluefin tuna (*Thunnus maccoyii*) to a single meal with an emphasis on volume of feed ingested, dietary energy and baitfish size

This Trial involved analysis of archival data and identification of relationships between dietary energy on rate and duration of visceral warming, time to reach maximum visceral warming (t_{max}), an indicator of visceral warming (Feed Measure – FM) and visceral warming in response to the size of baitfish offered. The Trial was designed to assess visceral warming responses to differences in ration, and energy content of different baitfish fed to SBT. Baitfish differed markedly in lipid content which explains the large differences in energy content (Table 3.1.1). A total of 76 records from 130 total records were used in this Trial.

The water temperature (T_w) range during the Trial reflected lower winter temperatures (the lower end of a winter T_w range) that ranged between 13.4^{o}C and 16.3^{o}C. Water temperature at the beginning of the Trial was 14^{o}C, dropping to 13.4^{o}C after the first two weeks and then steadily rising to 16.3^{o}C by the end of the Trial (Figure 3.1.1).

The typical visceral warming responses of SBT fed diets comprising different energy levels are reflected in the results of *Archtag* software analysis (Figure 3.1.2, Figure 3.1.3, Figure 3.1.4 and Figure 3.1.5). In general, it appears that the lower the energy diet consumed relates to a quicker response for visceral temperature (T_{vis}) to reach maximum visceral temperature (T_{max}) and shorter time taken to return to basal temperature (T_b).

There was a significant difference between the feed measure (FM) mean values for the different baitfish ($F = 5.047$, $df = 3.72$, $p = 0.003$). The mean FM values were ordered according to the total energy contents of the baitfish (Figures 3.1.6).

The mean FM for *S. sagax* (US) (5183.7) was the highest, followed by *S. sagax* (EC) (4676.4), *S. pilchardus* (4405.2), and *S. sagax* (PL) had the lowest mean FM value (3619.8) and the lowest corresponding total energy content. These results strongly suggest that the energy content was a key predictor of FM and was explored in Trial 2.

Table 3.1.1 Mean baitfish size, proximate values and energy content (±SE), for baitfish n=4 used in Trial 1.

Feed Type	Weight (g)	Protein g/100 g	Lipid g/100 g	Moisture g/100 g	Energy kJ/100 g
S. sagax (US)	100.08 ±0.5	17.87 ±0.09	15.51 ±0.32	63.15 ±0.29	886 ±10.65
S. sagax (EC)	37.60 ±0.26	18.74 ±0.35	5.96 ±0.06	71.48 ±0.20	558 ±4.41
S. pilchardus	50.41 ±0.49	16.98 ±0.06	3.05 ±0.08	76.11 ±0.13	416 ±2.11
S. sagax (PL)	26.93 ±0.46	17.69 ±0.17	1.61 ±0.05	76.46 ±0.10	383 ±3.65

Note - (US) sourced from the United States of America, (EC) sourced from the East Coast of Australia, and (PL) sourced from Port Lincoln, Australia

Relationship between FM and dietary energy of different baitfish

A General Linear Model (GLM) was used to predict FM from dietary energy (kJ). Feed type was also included in the model. There was a significant effect of both dietary energy (kJ) ($F = 507.852$, $df = 1, 71$, $p < 0.001$) and feed type ($F=13.633$, $df = 3, 71$, $p<0.001$) on FM. Specifically, with diet energy held constant as a covariate, the marginal mean of the baitfish *S. sagax* (US) was significantly different ($p< 0.001$) to all other types of baitfish used in this Trial. This suggests that *S. sagax* (US) had a different relationship between dietary energy and food measure than the other three feed types.

A new GLM model specifying the addition of an interaction term (i.e. multiplicative effect between feed type and dietary energy) removed the main effect of baitfish type but revealed a statistically significant interaction between dietary energy and food type ($F = 2.998$, $df = 3,68$, $p = 0.037$). A significant interaction means that the slope of the continuous

variable (in this case, dietary energy) is different for one or more levels of the categorical variable (i.e. feed type). The interaction parameter estimate (i.e. beta weight) for *S. sagax* (US) was the only one that was statistically significant (p = 0.023). Thus, the baitfish *S. sagax* (EC)*, S. sagax* (PL) *and S. pilchardus* were pooled because there was no significant statistical difference between their respective results in their relationship between dietary energy and FM. The relationship between dietary energy and FM was modelled separately for *S. sagax* (US).

Figure 3.1.7 shows the individual regression formula for the prediction of FM from dietary energy for each of the baitfish while Figure 3.1.8 shows the pooled regressions (with *S. sagax* (US) modelled separately). The pooled baitfish results provide the following equation and coefficient $Y = 0.777x + 1722.8$ and $R^2 = 0.829$ whereas *S. sagax* (US) had the following equation and R^2 value $Y = 0.661x + 1311.6$; $R^2 = 0.954$. The high R^2 values for the GLM for *S. sagax* (US) and pooled baitfish demonstrated dietary energy accounted for a large amount of the variation in FM. The implications of these results are that the rate of visceral warming was strongly affected by the energy content of the feed consumed; and it was possible to predict warming by knowing the energy content of the type of baitfish fed.

Relationship between combined data for FI and dietary energy

To further understand whether visceral warming was influenced by dietary energy regardless of bait type, all data points were pooled and analysed by applying a GLM. Despite the differences in slope for the high energy baitfish, there remained a large, significant effect in the pooled data demonstrating that visceral warming (FM) was influenced by dietary energy ($F=389.305$, $df = 1, 74$, $p<0.001$).

The GLM values for the combined data demonstrated a very strong linear regression relationship $Y = 0.6045x + 2167.9$; $R^2 = 0.84$.

Figure 3.1.9 shows the relationship between FI and dietary energy was not strictly linear. When the energy content of feed consumed approached 8000 kJ there was little change to the feed measure above 7000 suggesting there was some kind of visceral warming control mechanism in place. To understand this process a two point polynomial regression analysis was applied to the data points because it allows the data points to be smoothed to reflect

changes between the points within the range of difference. This resulted in a stronger regression R^2 value with the following equation:

Y=*-6E-05x^2+1.2179x+971.18; R^2 = 0.912* (Figure 3.1.9).

This highlights that visceral warming may reach a plateau which was reflected in the FM and this level will be maintained independent of energy value of the diet above 8000 kJ until the feed has been digested. These results should be viewed as preliminary given that there were few data with energy values above this range, and all were of the *S. sagax* (US) feed type.

Relationship between duration of visceral warming and different baitfish

A GLM was used to predict Feed Duration (FD) from dietary energy (kJ). Feed type was also included in the model. There was a significant effect of dietary energy (F = 100.547, df = 1, 71, p <0.001). No effect was seen for feed type (p = 0.104) on feed duration. Confirmation of homogeneity of slopes was indicated by replication of the analysis with the addition of an interaction term between dietary energy and feed type to the model. The interaction was not significant (p = 0.450) indicating that the relationship between dietary energy and FD was consistent across baitfish types. Thus, the baitfish types were pooled. The individual regression lines for each baitfish type are show in Figure 3.1.10.

The regression of FD on dietary energy for the pooled data was statistically significant (F = 149.749, df = 1.74, p < 0.001) with the resulting regression equation *Y = 0.1512x + 875.9, R^2 = 0.669* (Figure 3.1.11). This was not as precise a relationship as that seen between dietary energy and FM (above). Investigations were conducted to determine whether a polynomial effect provided better prediction than a linear model. However, the quadratic term was not statistically significant, (p = 0.183 for quadratic term), indicating that the linear relationship provided the best fit to the data. These results indicate that higher energy diets influence the duration of visceral warming in a linearly fashion.

Relationship between time taken to reach peak visceral warming (t_{max}) and intake of baitfish

A GLM was used to predict t_{max} from feed intake. Feed intake (FI) was calculated as a percentage of body weight. Feed type was also included in the model. There was a

significant effect of both FI (F = 62.326, df = 1, 71, p <0.001) and feed type (F=47.034, df = 3, 71, p<0.001) on t_{max}. Specifically, with FI held constant as a covariate, the marginal means of each baitfish were significantly different from one another ($p < 0.05$), with the exception that *S. sagax* (EC) and *S. sagax* (PL) did not differ significantly ($p = 0.09$). This suggests that a different relationship between FI and t_{max} was indicated for the different baitfish. A new GLM model specifying an interaction term removed the main effect of baitfish, but revealed a statistically significant interaction between FI and bait type ($F = 29.309$, df = 3,68, $p < 0.001$). This indicated heterogeneity of slopes, meaning that a different relationship between FI and t_{max} was seen for the different baitfish types. Thus, they were modelled separately.

The relationships between FI and t_{max} for each baitfish are shown in Figure 3.1.12 and equations are listed in Table 3.1.2. The magnitude of the slopes increases with dietary energy of the baitfish, and the strength of the relationships (R^2) also increases with increasing dietary energy. Thus, the higher the dietary energy, the stronger the prediction of t_{max} from FI.

Table 3.1.2 GLM equations and R^2 values for prediction of t_{max} from FI for feed types used in Trial 1.

Feed Type	Equation	R^2 value
S. sagax (US)	y = 386.72x + 55.586	0.87
S. sagax (EC)	y = 179.86x + 92.508	0.631
S. pilchardus	y = 65.753x + 214.38	0.37
S. sagax (PL)	y = 36.291x + 320.39	0.062

Summary

The results of this Trial strongly suggest dietary energy content was a key predictor of FM. The implications of these results are that the rate of visceral warming was strongly affected by energy content of feed consumed, and it was possible to predict warming by knowing energy content of type of baitfish fed. The relationship between FM and dietary energy was not strictly linear. When the energy content of feed consumed approached 8000 kJ there was little change to the feed measure above 7000 suggesting some kind of visceral warming control mechanism. A two point polynomial regression analysis applied resulted in a stronger regression R^2 value with the following equation $Y=-6E-05x^2+1.2179x+971.18$; $R^2 = 0.912$ highlighting that visceral warming may reach a plateau and will be maintained independent of energy value of the diet. Furthermore, the results indicate that higher energy diets influence the duration of visceral warming in a linearly fashion.

The R^2 values for the relationships between FI and t_{max} increased with increasing dietary energy. Thus, the higher the dietary energy, the stronger the prediction of t_{max} from FI.

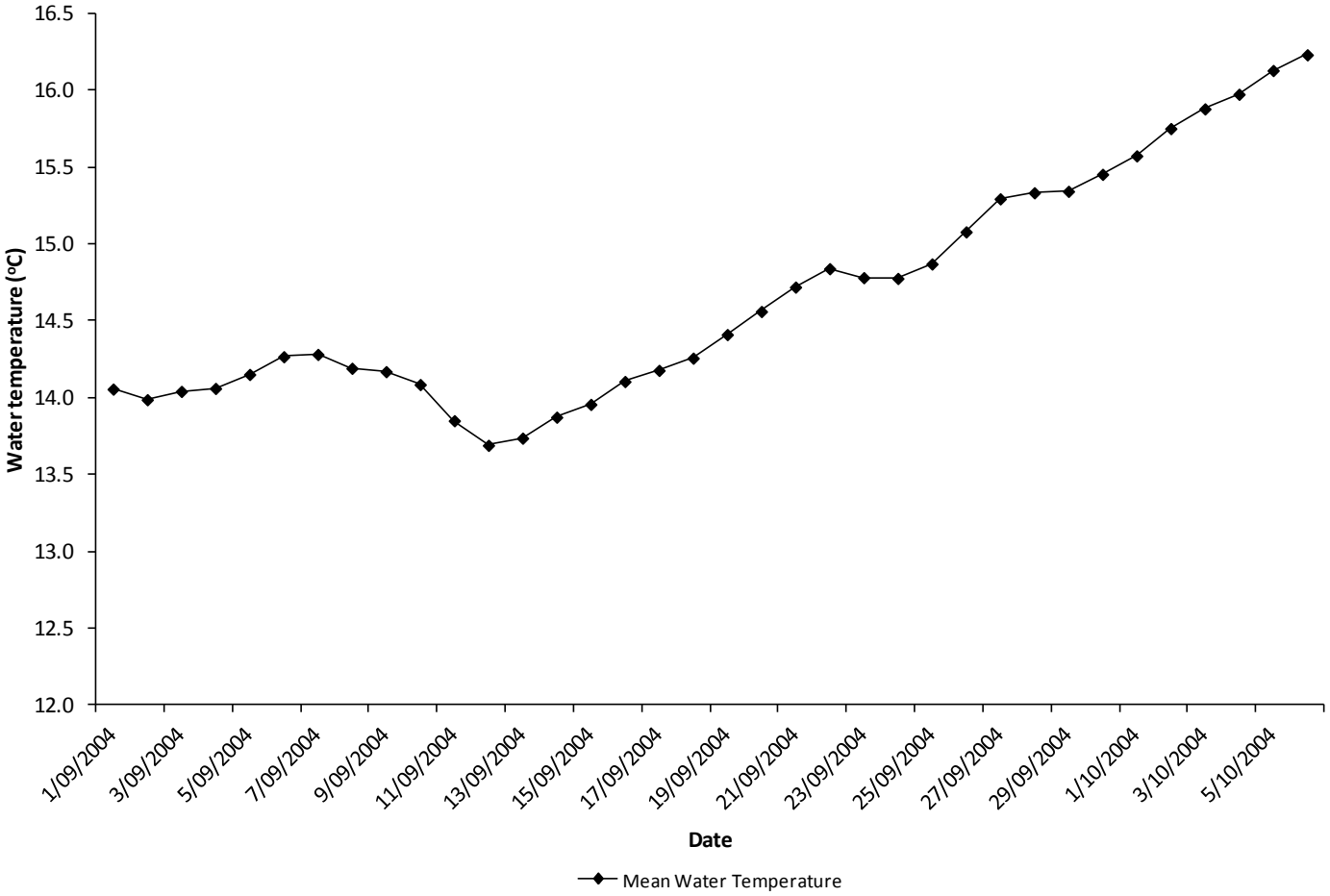


Figure 3.1.1 Mean daily water temperature (^{o}C) during the experimental period (date) measured at a depth of 5m using a Vemco data recorder.

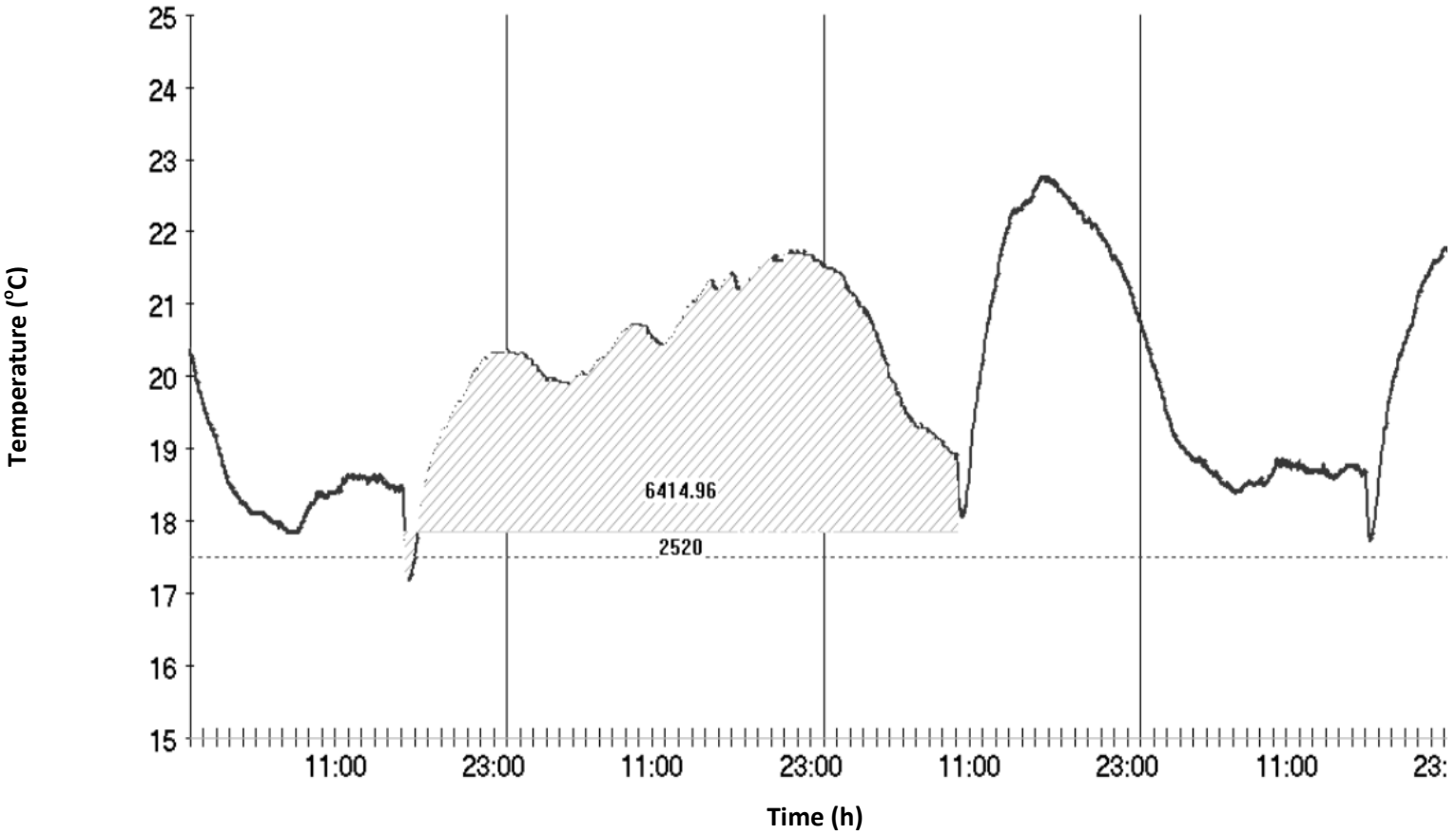


Figure 3.1.2 SBT visceral warming pattern expressed as a function of temperature area under the curve (FM) in relation to time (h) to approximately 1 kg of high lipid baitfish *S. sagax* (US).

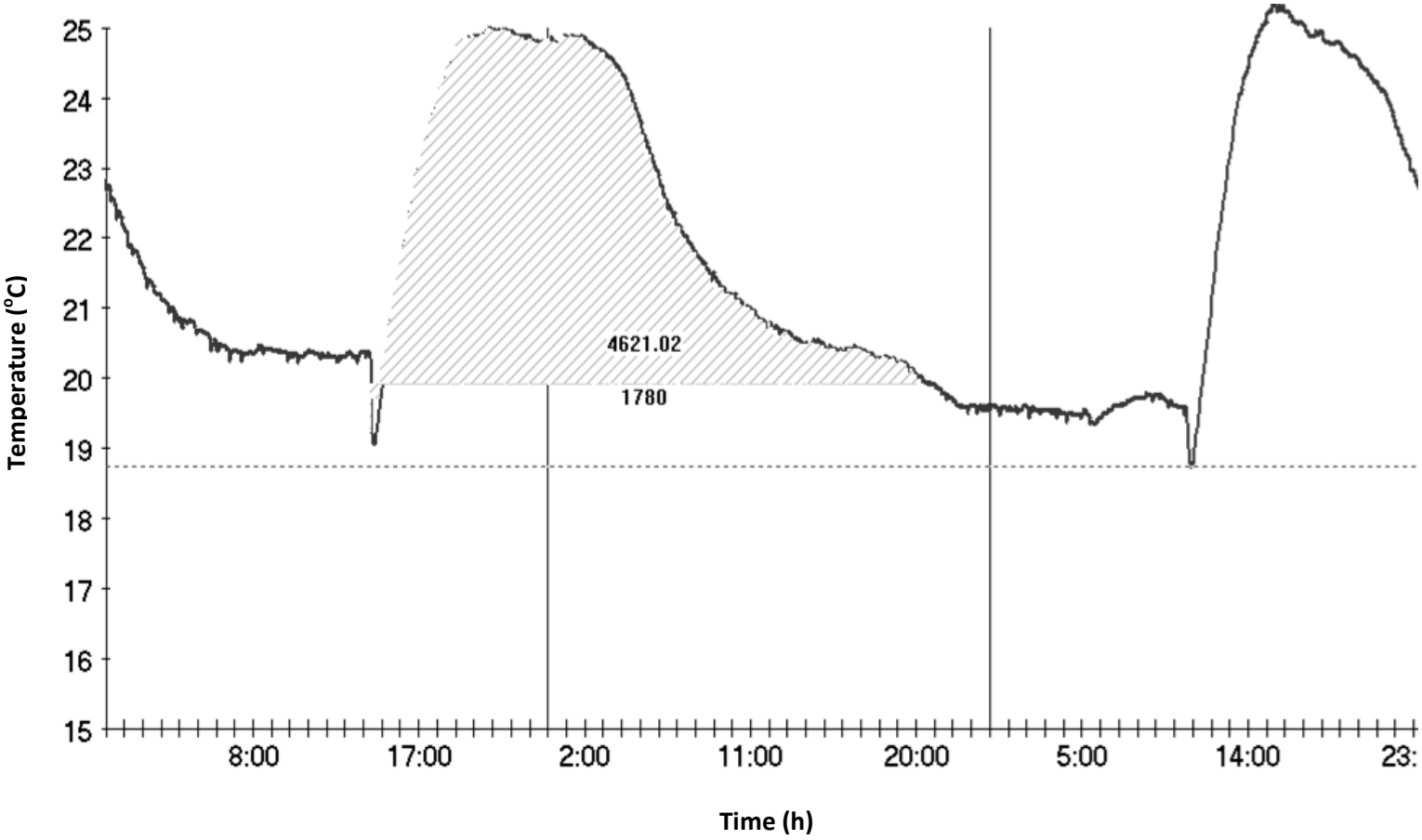


Figure 3.1.3 SBT visceral warming pattern expressed as a function of temperature area under the curve (FM) in relation to time (h) to approximately 1 kg of medium lipid baitfish *S. sagax* (EC).

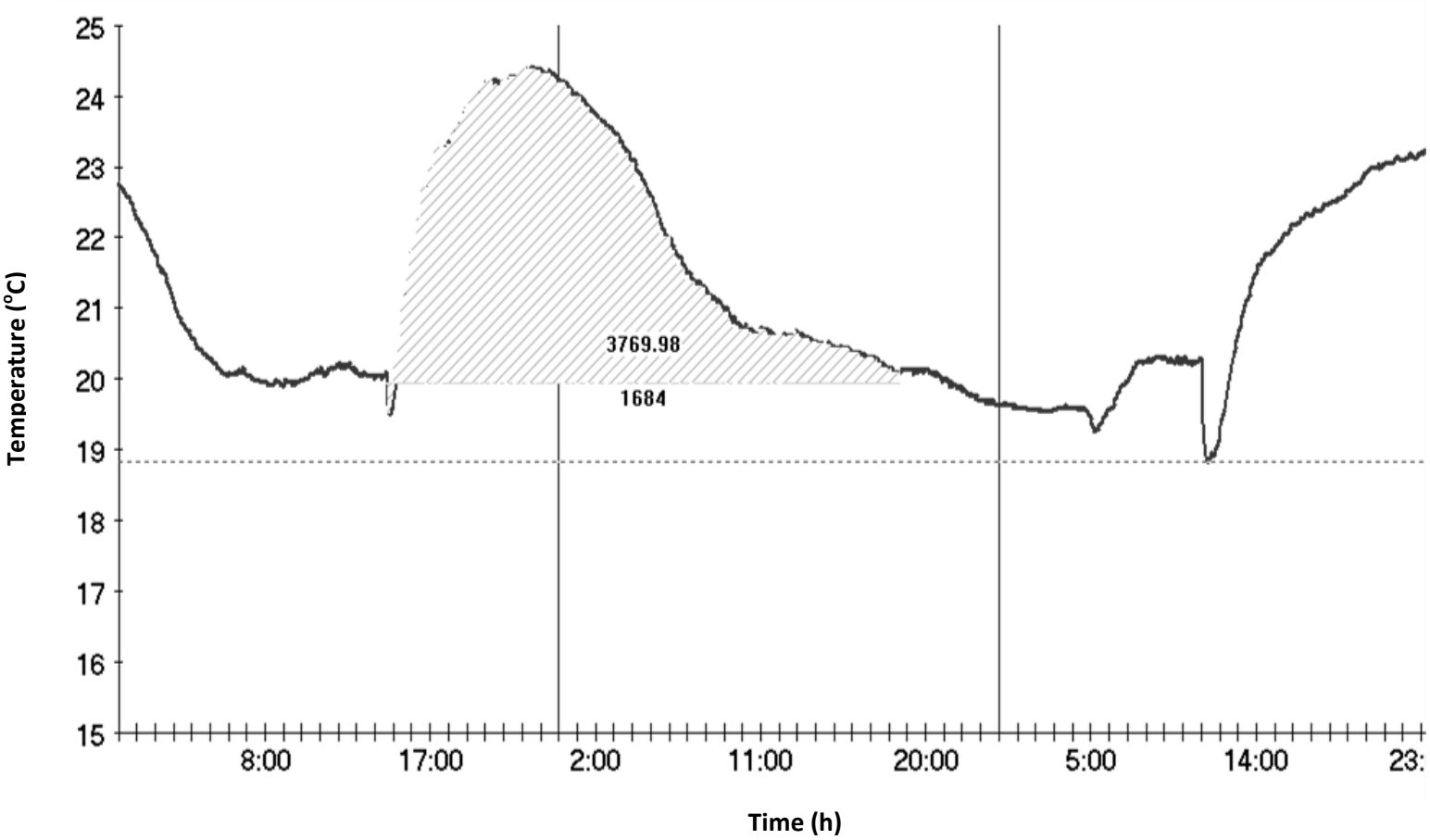


Figure 3.1.4 SBT visceral warming pattern expressed as a function of temperature area under the curve (FM) in relation to time (h) to approximately 1 kg of low lipid baitfish *S. pilchardus*.

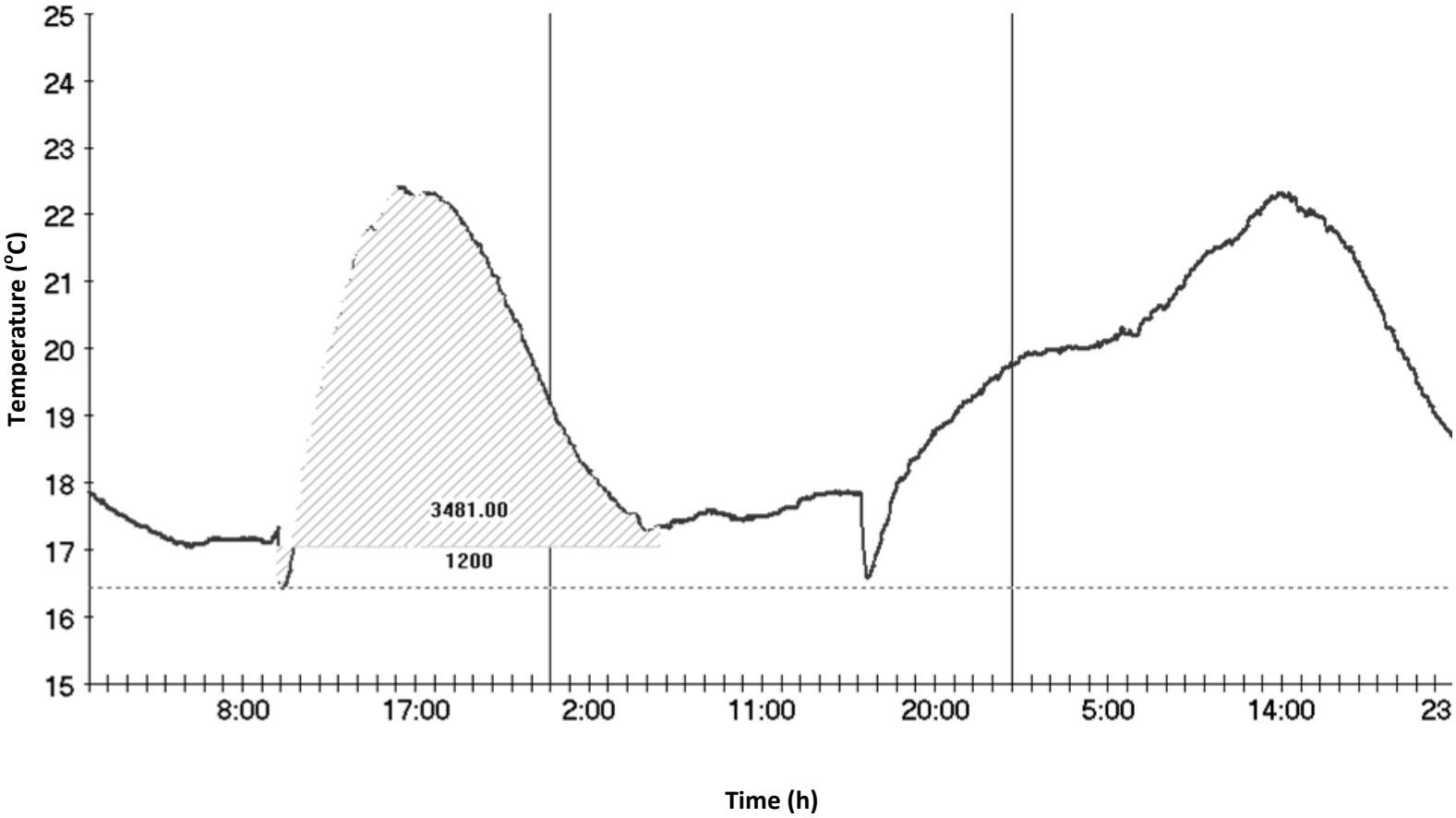


Figure 3.1.5 SBT visceral warming pattern expressed as a function of temperature area under the curve (FM) in relation to time (h) to approximately 1 kg of low lipid baitfish *S. sagax* (PL).

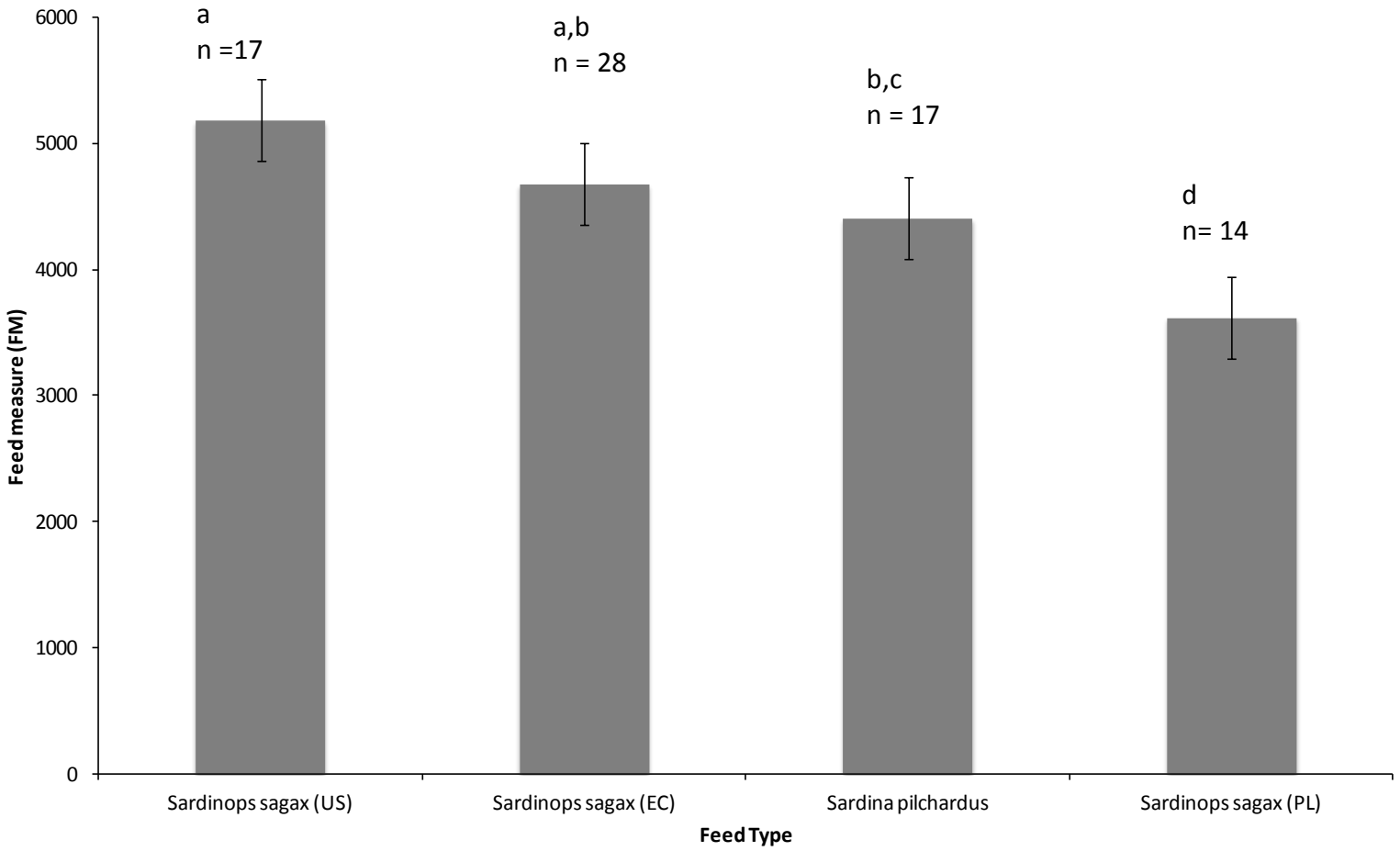


Figure 3.1.6 The relationship between mean feed measure (FM) and baitfish types used in this study. Data are presented as mean, standard error. Means with no common letters are significantly different.

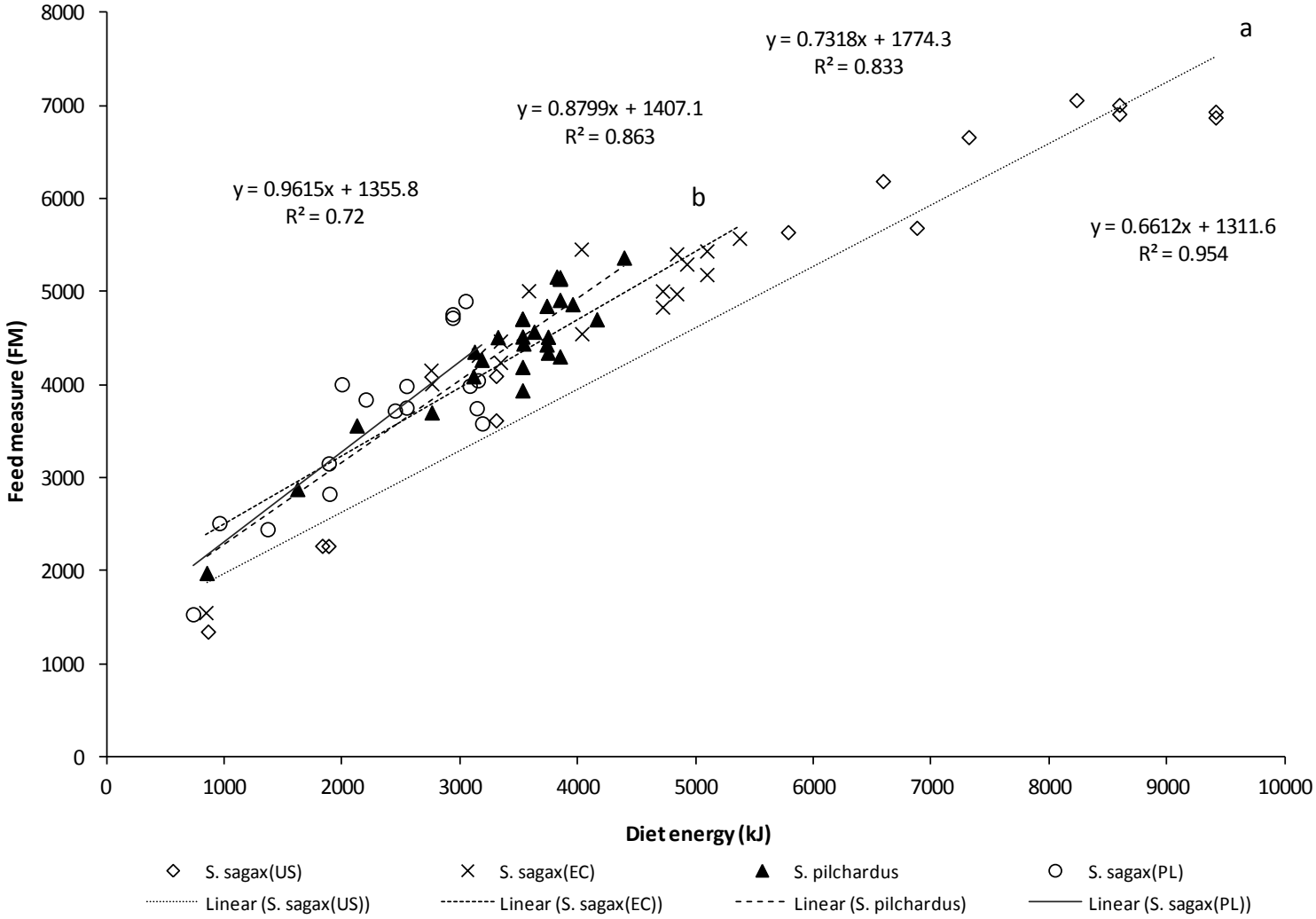


Figure 3.1.7 General linear regression analysis of the relationship between feed measure (FM), dietary energy (kJ) and baitfish types. Slopes with no common letters are significantly different.

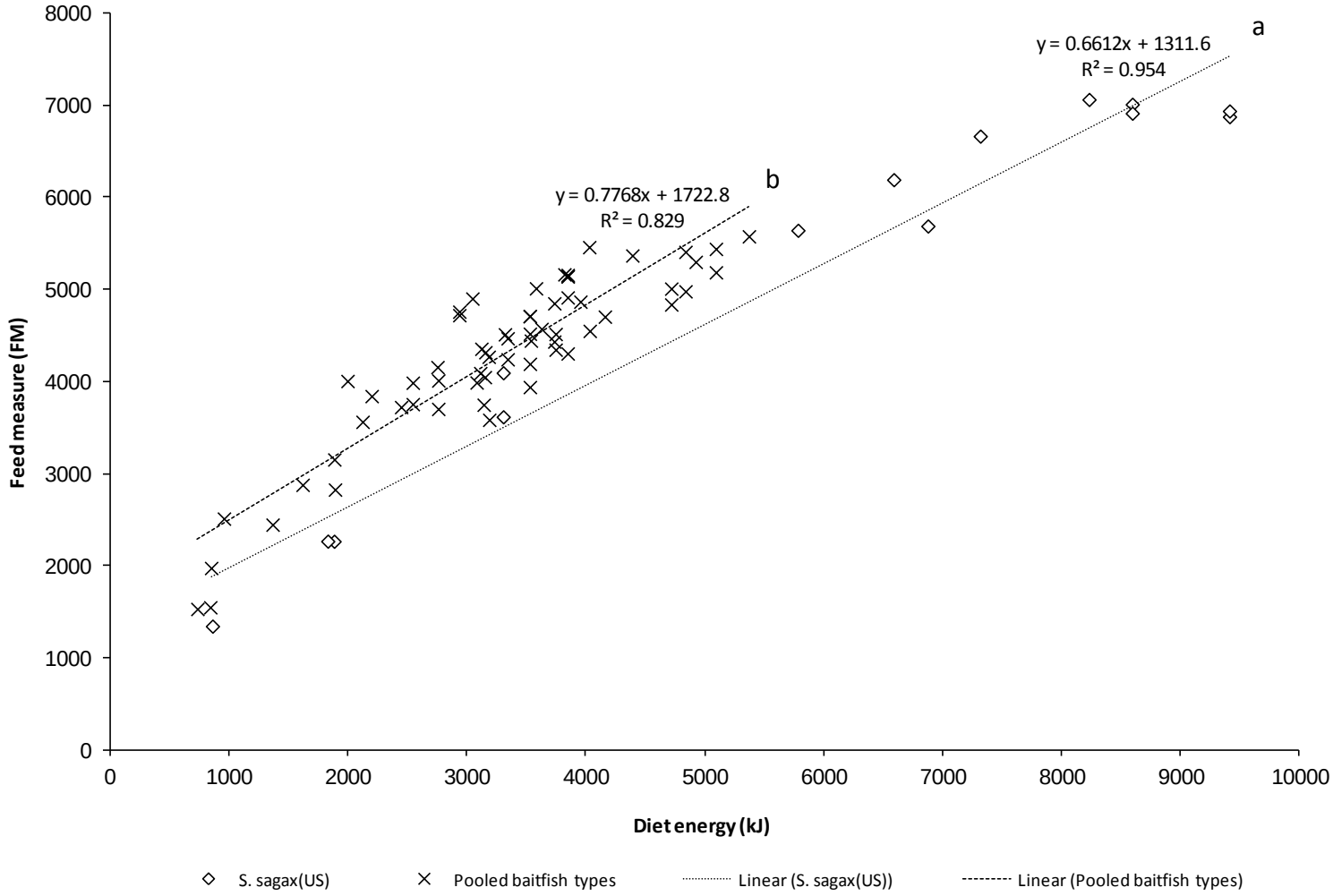


Figure 3.1.8 General linear regression of the relationship between feed measure (FM) and dietary energy (kJ) for *S. sagax* (US) and pooled baitfish types *S. pilchardus, S. sagax* (EC), and *S. sagax* (PL). Slopes with no common letters are significantly different.

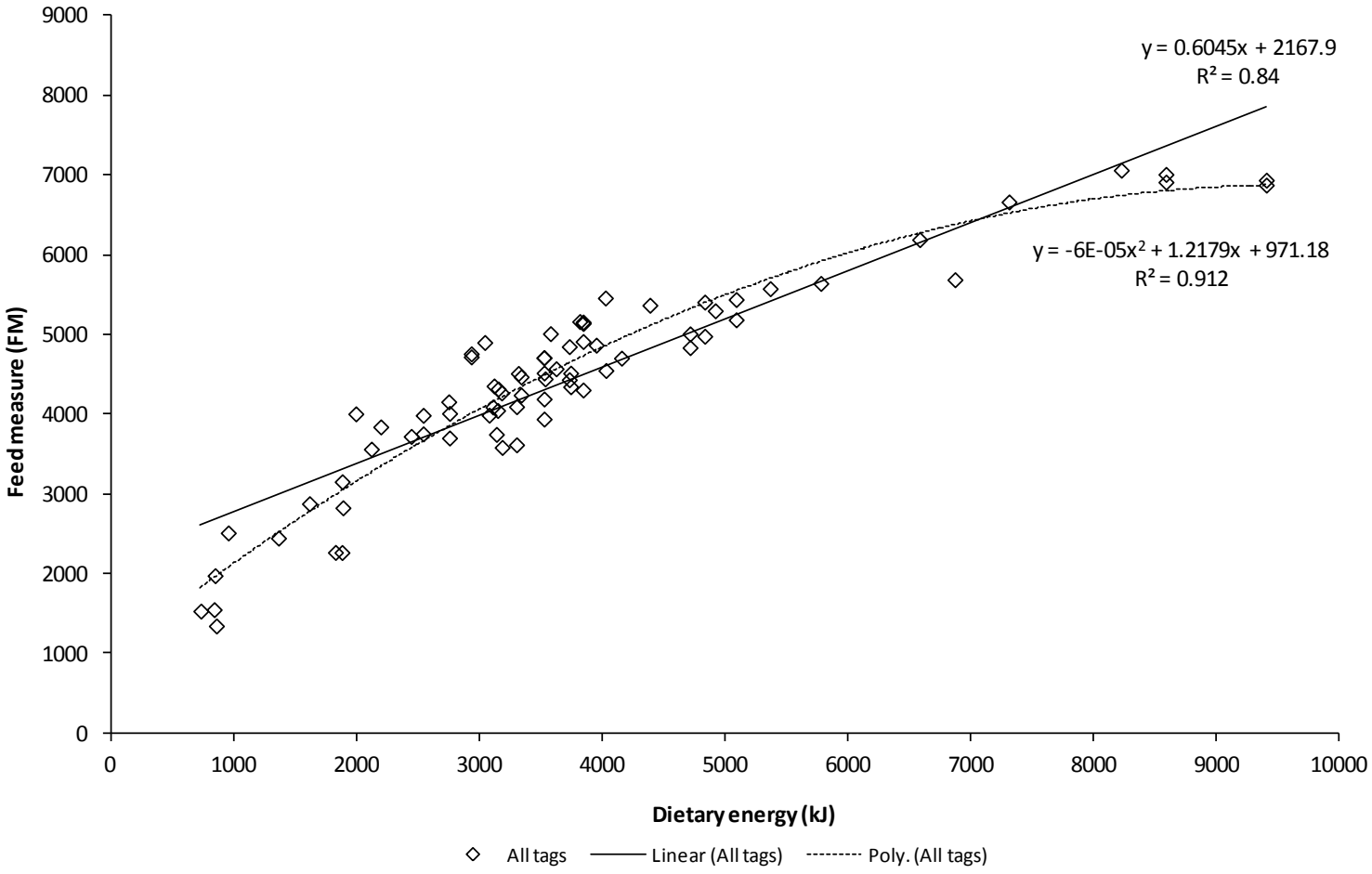


Figure 3.1.9 General linear regression and two point polynomial regression analysis of the relationship between feed measure (FM) and dietary energy (kJ) for pooled baitfish types.

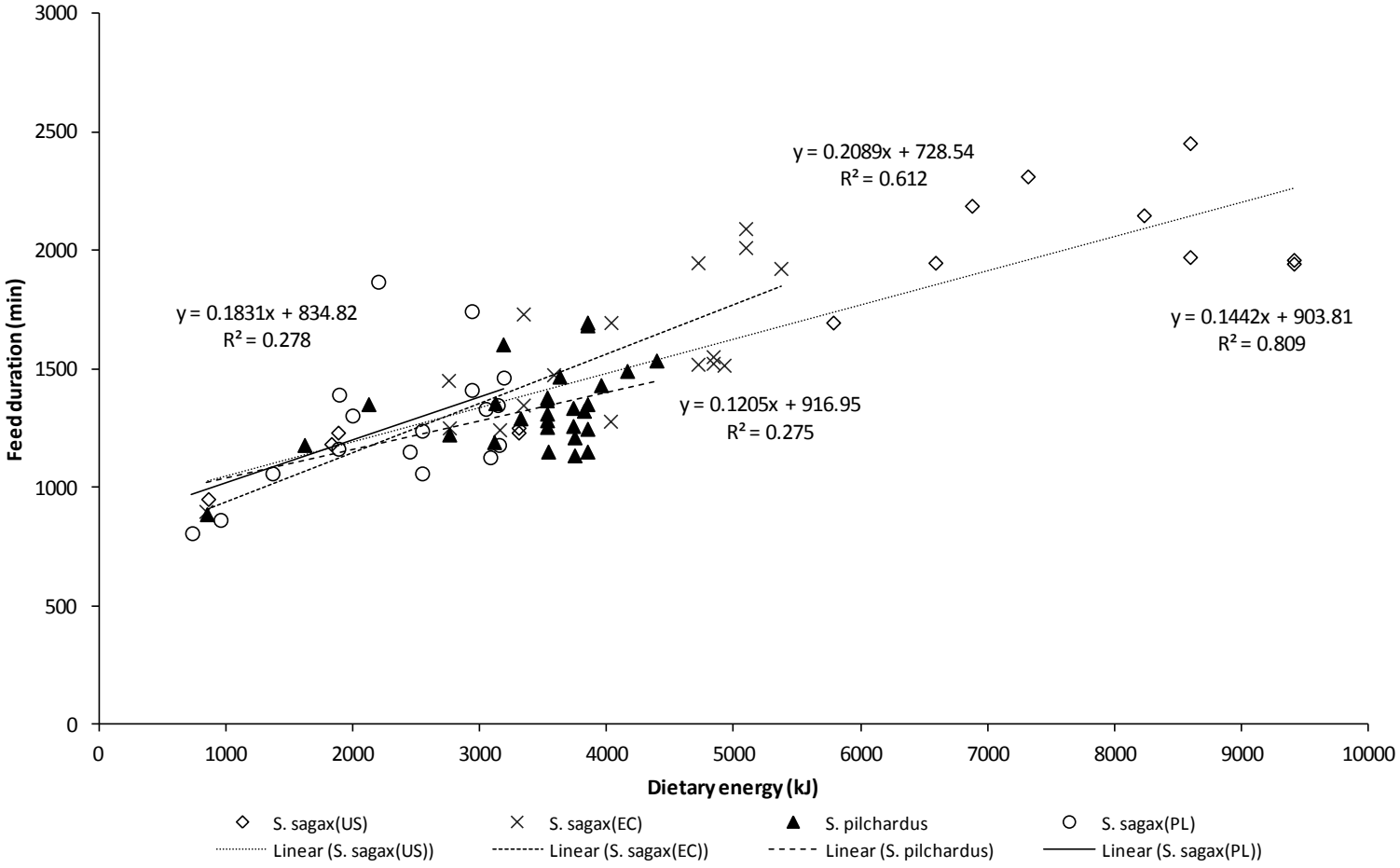
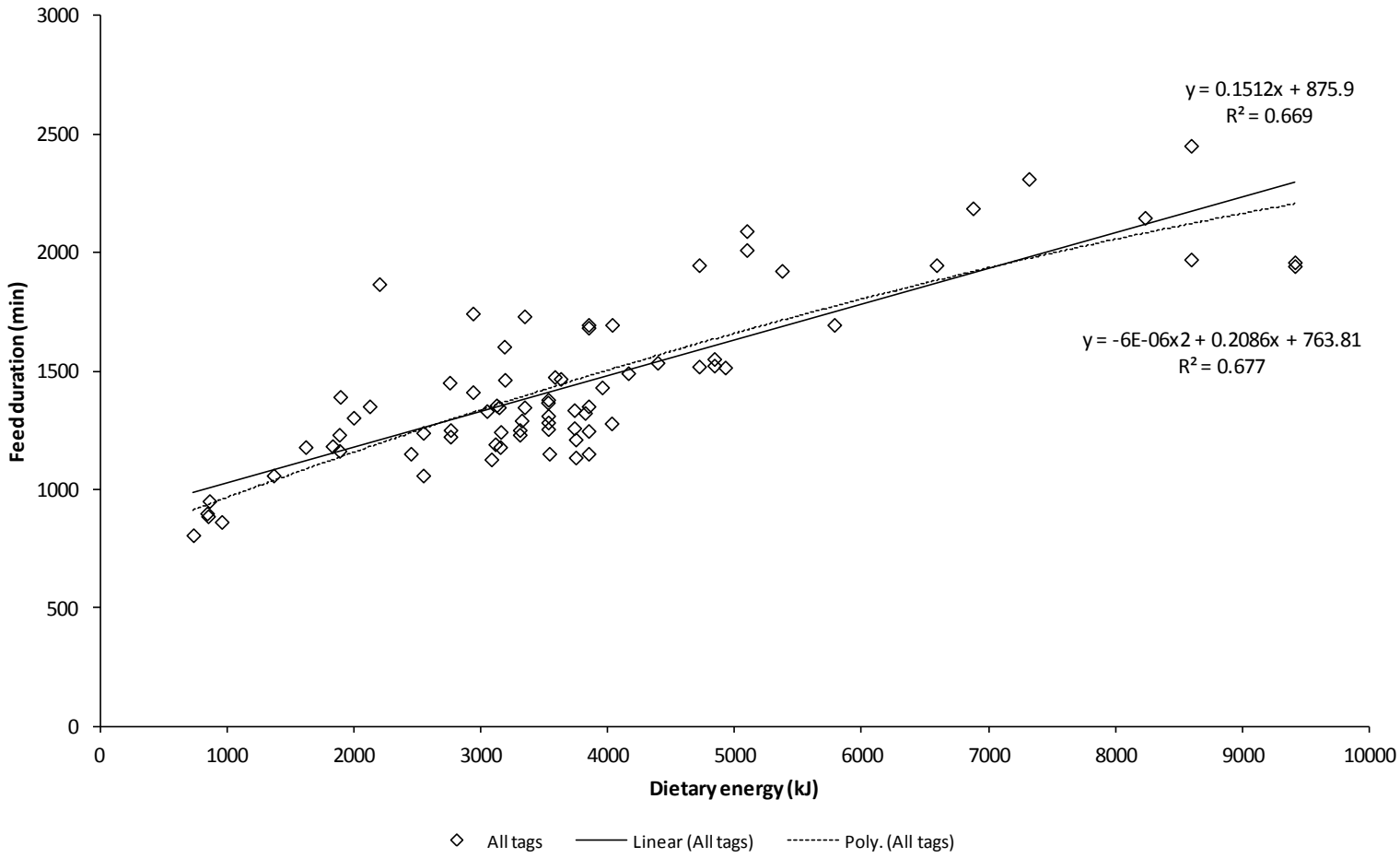


Figure 3.1.10 General linear regression analysis of the relationship between feed duration (min), dietary energy (kJ) and baitfish type.



+

Figure 3.1.11 General linear regression and two point polynomial regression analysis of the relationship between feed duration (min) dietary energy (kJ) and pooled data.

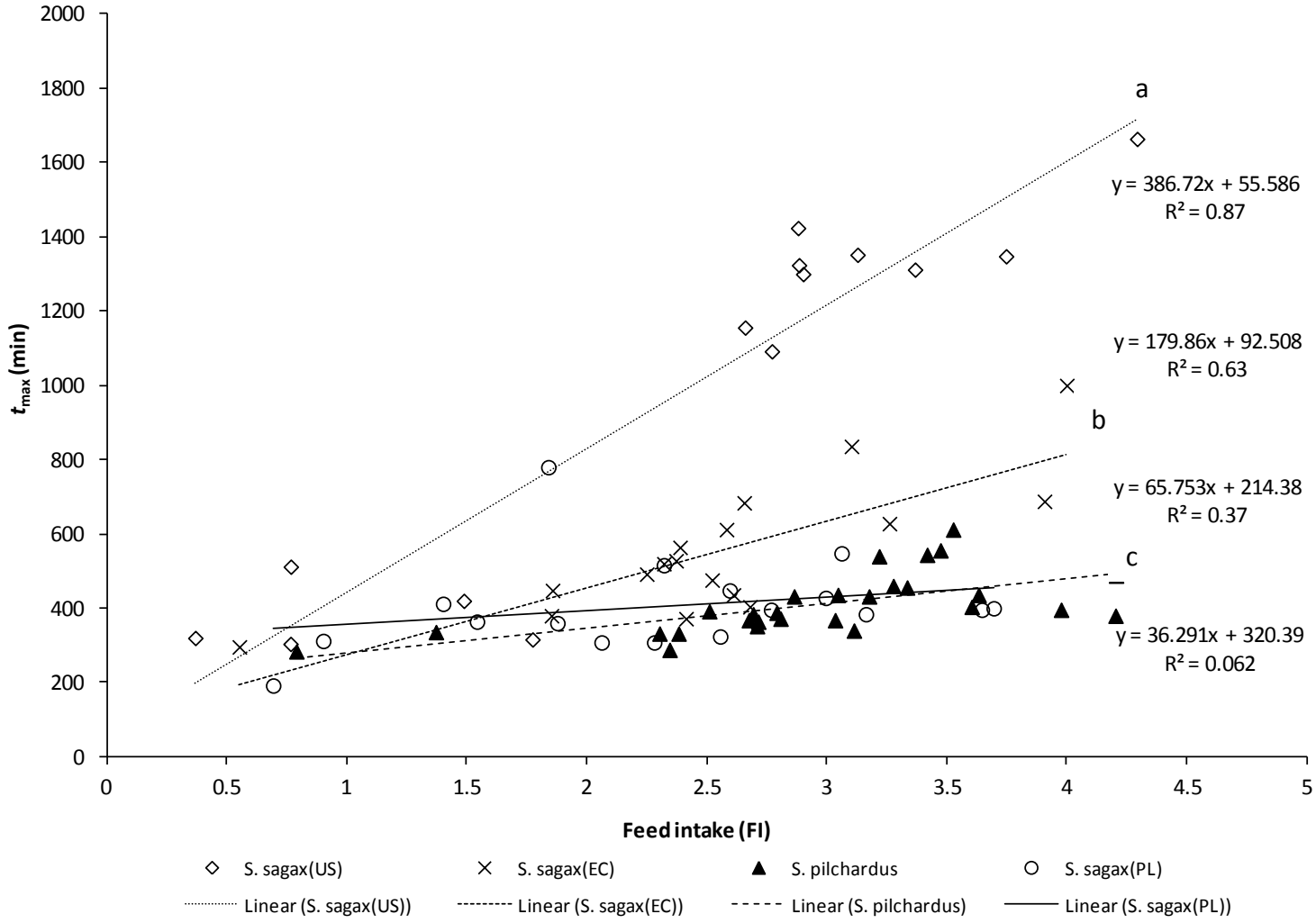


Figure 3.1.12 General linear regression analysis of the relationship between t_{max} (min) feed intake (FI) consumed expressed as % body weight per day and baitfish types. Slopes with no common letters are significantly different.

3.2 Trial 2 – The visceral warming response to one, two or three feeds in southern bluefin tuna (*Thunnus maccoyii*) with an emphasis on weight of feed ingested and dietary energy at two different water temperatures

Trial 1 investigated the influences on visceral warming patterns when SBT were fed a single meal. The data obtained from Trial 1 suggested that dietary energy has a significant effect on visceral warming and that there appears to be a level where visceral warming plateaus suggesting a control mechanism. Trial 2 built on the results of Trial 1 by analysing archival tag data from SBT that have been fed one, two or three times per day with baitfish of the same dietary energy in cool and warm water.

Period 1 of Trial 2 occurred between 9 November and 6 December 2006, during which a total of 211 records were obtained. Of these records 41 were analysed. Period 2 of this Trial occurred between 7 February and 5 March 2007 during which 162 records were obtained. Of these records 36 were analysed. The loggers failed to record after 28 February as they ran out of memory space.

Water temperature (T_w) during Period 1 of the Trial steadily increased from 17^{o}C at the beginning of the Trial to 19^{o}C at the conclusion of the first part of the Trial. At the beginning of Period 2 of the Trial, T_w was 22^{o}C, and peaked above 24^{o}C during the Trial (Figure 3.2.1).

There was very little difference in the nutritional composition of the sardines used in Period 1 and 2 of this Trial, however, there was a slight difference in the individual weight of the baitfish used (Table 3.2.1).

Table 3.2.1 Mean baitfish size, proximate values and energy content with ± SE for feed types used in Trial 2.

Feed Type *S. sagax*	**Weight (g)**	**Protein g/100 g**	**Lipid g/100 g**	**Moisture g/100 g**	**Energy kJ/100 g**
Period 1	53.10	19.18	6.33	69.89	571
November 2006	±1.52	±0.05	±0.06	±0.14	±1.58
Period 2	32.75	20.31	6.00	70.26	568
February 2007	±0.09	±0.04	±0.00	±0.03	±0.37

Relationship between feed measure (FM), feed frequency, and dietary energy (kJ)

Trial 2: Period 1

Significant differences in FM means were seen for feed frequencies of one, two, or three times/day ($F = 11.635$, $df = 2, 38$, $p < 0.001$). Inspection of the means (Figure 3.2.2) shows that the FM was much higher at three feeds per day than at either one or two feeds per day. Post-hoc testing revealed that differences were seen between FM values at three feeds per day ($p < 0.001$) compared to values at one or two feeds per day, which did not differ from one another. However, importantly, the total energy consumed (also shown in Figure 3.2.2) was almost identical to the FM values at each feed frequency. Thus, it was highly plausible that the differences in FM at the different feed frequencies were related to the energy consumed, rather than the frequency.

The likelihood that differences in FM were related more to the energy consumed, rather than feed frequency, was investigated using a GLM predicting FM from both feed frequency and dietary energy. There were significant effects of both dietary energy ($F = 144.655$, $df = 1, 37$, $p < 0.001$) and feed frequency ($F = 12.954$, $df = 2, 37$, $p < 0.001$). In other words, different marginal means of FM were seen at different feed frequencies. Therefore, there was evidence that differences in the FM were based on feed frequency even when holding constant the dietary energy consumed. The interaction between dietary energy and feed

frequency revealed significant effects for each level of the feed frequency variable ($p < 0.05$).

Thus, not only did the intercepts differ, but the slopes between FM and dietary energy differed according to one, two, or three feeds per day. Therefore, these were modelled separately.

The resulting regression lines of FM on dietary energy are shown in Figure 3.2.3 and equations are listed in Table 3.2.2. The intercepts increased in conjunction with the number of feeds. A linear pattern was not evident for the slopes however, as the slope for two feeds per day was the highest of the three. Overall, it appeared that the relationships between FM and dietary energy were strongest when there were fewer feeds – the R^2 for one to two feeds per day were both over 90%. The relationship was somewhat diminished in the group with three feeds per day (and a correspondingly higher dietary energy intake).

Table 3.2.2 GLM equations and R^2 values for prediction of FM from dietary energy based on the number of feeds for Trial 2: Period 1 and 2.

Feed frequency (number of feeds per day)	**Equation and R^2 value Period 1**	**Equation and R^2 value Period 2**
1x	y = 0.6796x + 501.77	y = 0.1509x + 2060
	R^2 = 0.919	R^2 = 0.656
2x	y = 0.7477x + 820.3	y = 0.2241x + 1694.5
	R^2 = 0.958	R^2 = 0.732
3x	y = 0.3988x + 2343.9	y = 0.1991x + 1147.3
	R^2 = 0.515	R^2 = 0.94

Trial 2: Period 2

The same analysis between FM, feed frequency, and dietary intake was repeated on the data obtained in Period 2 of this Trial. As with Period 1, significant differences were seen in the mean FM values according to feed frequency ($F = 5.417$, $df = 2, 33$, $p = 0.009$). However, as seen in Figure 3.2.4, the means showed a different pattern. Mean FM values for one feed

(3034.68) or two feeds (3030.61) were almost identical. The mean FM value for three feeds (2247.93) was significantly lower (< 0.01) than the FM means for one or two feeds. This was in contrast to Period 1, in which the mean FM for three feeds per day was significantly higher than the FM means at one or two feeds per day. Furthermore, whereas the FM values were overlapping the total energy values in Period 1, mean dietary energy values were considerably higher than the FM in Period 2 (Figure 3.2.4).

Comparison of Figures 3.2.2 and 3.2.4 reveals that the total energy consumed was considerably higher in Period 2 than in Period 1. It should be noted that the number of data points for three feeds (n=6) was lower than either one or two feeds (n=11 and n=19, respectively), and therefore the results were interpreted with caution.

A General Linear Model (GLM) analysis of the effects of both feed frequency and dietary energy on FM revealed significant effects for both. Dietary energy was a significant predictor of FM ($F = 83.321$, $df = 1, 32$, $p < 0.001$) as was feed frequency ($F = 13.807$, $df = 2, 32$, $p < 0.001$). Therefore, even with dietary energy held constant between groups, significant differences in mean FM values (intercepts) were seen according to the feed frequency. The possibility of differences in slopes at the different feed frequencies was investigated by adding an interaction term between dietary energy and feed frequency to the model predicting FM. A significant interaction term would suggest that the slopes between dietary energy and FM differed according to one or more levels of the categorical variable (i.e. feed frequency). However, the addition of an interaction term was not statistically significant ($p = 0.331$), and the individual interaction parameters were similarly non significant. Thus the relationship between FM and total dietary energy did not differ significantly according to the number of feeds, although the marginal mean values (intercepts) did differ. Therefore, the data could be pooled to provide an overall estimate of FM from dietary energy to apply across number of feeds. The individual regression lines are shown in Figure 3.2.5 and the pooled data in Figure 3.2.6. The equation for the pooled data was $Y = 0.2066x + 1653$, $R^2 = 0.611$. A two point polynomial regression analysis was applied to the data points because it allows the data points to be smoothed to reflect changes between the points within the range of difference. This resulted in a slightly stronger regression R^2 value with the following equation: $y=-2E\text{-}05x^2+0.4615x+912.68$; $R^2 = 0.667$ (Figure 3.2.6).

Relationship between peak visceral warming (t_{max}), feed frequency, and intake of baitfish (FI)

Figures 3.2.7 and 3.2.8 visually show visceral warming patterns of SBT fed two or three times per day. These figures provide an indication of the difficulty of predicting feed intake using t_{max} as results can change depending on the individual feeding behaviour of SBT and the amount a SBT consumes at a given meal.

Trial 2: Period 1

There was a significant difference in the mean t_{max} scores according to feed frequency ($F = 7.745$, $df = 2, 38$, $p = 0.002$). Post-hoc analyses showed that the means for one and three feeds per day differed from one another ($p < 0.001$), while the mean for two feeds per day did not differ from the other values. The mean t_{max} and FI scores (Feed weight / Initial body weight * 100) according to feed frequency are shown in Figure 3.2.9. There appeared to be a linear increase in t_{max} according to the number of feeds per day. However, FI did not show a linear pattern according to the number of feeds. Intake was relatively constant for one and two feeds, and considerably higher for three feeds per day.

A GLM was used to predict t_{max} from FI and feed frequency and attempt to clarify the relationship between these variables. With both feed frequency and FI as predictors, there was a significant effect of feed frequency on t_{max} ($F = 5.408$, $df = 2, 37$, $p = 0.009$) but the relationship of FI was not statistically significant ($F = 2.200$, $df = 1, 37$, $p = 0.147$). This indicates that with FI held constant, there remained a difference in the mean t_{max} values according to the number of meals per day. t_{max} was lowest for one feed per day and highest for three feeds per day, independent of FI. However, importantly FI was not a significant predictor of t_{max} in this equation.

The very poor linear relationships (Figure 3.2.10) between t_{max} and FI at each feeding frequency reaffirm the difficulty of applying t_{max} as a robust measure of intake due to individual SBT feeding behaviour. The R^2 values ranged from 0.05 to 0.16, indicating that only a small proportion of the variance in t_{max} could be explained by FI in this Trial. Results indicate that SBT used in this Trial were regulating t_{max} independent of FI which would suggest there was a physiology requirement and subsequent response to manage the rate and maximum temperature (T_{max}) of visceral warming.

Trial 2: Period 2

In Period 2 of the Trial, there was a marginally significant effect of feed frequency on t_{max} ($F = 3.071$, $df = 2, 33$, $p = 0.060$). Post hoc testing indicated that t_{max} for one and two feeds per day differed significantly ($p < 0.05$), whereas three feeds/ per day did not differ from the other two feed frequencies. As seen in Figure 3.2.11, the mean t_{max} value was highest for one feed per day.

The FI is also shown in Figure 3.2.12 and follows a similar pattern to t_{max} across the feed frequencies. That is, FI was highest for one feed per day and about equal for two to three feeds per day.

A GLM of the relationship between t_{max}, FI, and feed frequency revealed no significant associations. Treatment was a non-significant predictor of t_{max} ($F = 2.448$, $df = 2, 32$, $p = 0.102$) as was FI ($F = 0.589$, $df = 1, 32$, $p = 0.448$). Therefore, with FI held constant, no differences in t_{max} were seen according to the number of feeds per day. Furthermore, FI was not a reliable independent predictor of t_{max}. As seen in Figure 3.2.12, the slopes between t_{max} and FI were almost flat regardless of feed frequency. The R^2 values ranged from 0.01 to 0.04, indicating that almost no variance in t_{max} could be explained by FI.

Comparison between data obtained in Trial 2: Period 1 and Period 2

The relationships between FM and dietary energy obtained in Period 1 and Period 2 of this Trial were compared. t_{max} and FI were not assessed further given that the relationships were not significant in either part of the Trial.

Although different slopes were obtained in Period 1 of the Trial for the relationships between FM and dietary energy at different feeding events, the data were pooled in order to facilitate comparisons between sections. The pooled results for Period 1 were compared to the pooled results for Period 2. The GLM analysis revealed significant effects for dietary energy ($F = 85.964$, $df = 1, 74$, $p < 0.001$) and for the effects of period ($F = 39.941$, $df = 1, 74$, $p < 0.001$). In other words, dietary energy was a significant predictor of FM across Periods, yet the marginal means, or intercepts, of the two Periods differed. Re-analysis with inclusion of an interaction (i.e. multiplicative) term between dietary energy and Period indicated a significant interaction effect ($F = 60.663$, $df = 1, 73$, $p < 0.001$), and the main effect of Period (intercepts) remained statistically significant ($p = 0.004$). Both the slopes

and the intercepts of the regression lines differed according to Period (when the Trial was conducted).

The regression lines for each Period are shown in Figure 3.2.13.

For Period 1, the regression line was $y = 0.683x + 821.18$, $R^2 = 0.785$ and for Period 2, the equation was $y = 0.2066x + 1653$, $R^2 = 0.611$. Therefore, the prediction of FM from dietary energy was more precise in Period 1 than in Period 2.

Summary

In cool water the total energy consumed by SBT was almost identical to the FM values at each feed frequency. Thus, it was highly plausible that the differences in FM at the different feed frequencies were related to the energy consumed, rather than the frequency. A linear pattern was not evident for slopes of every feeding pattern but two feeds per day offered the strongest R^2 value.

The mean FM values in warm water were significantly lower than cooler water yet the mean dietary energy values were considerably higher than the FM suggesting heat conservation/expending mechanism.

The very poor linear relationships between t_{max} and FI at each feeding frequency reaffirm the difficulty of applying t_{max} as a robust measure of intake due to individual SBT feeding behaviour. It also appears that SBT used in this study were regulating t_{max} independent of FI which would suggest there was a physiology requirement and subsequent response to manage the rate and maximum temperature (T_{max}) of visceral warming.

Although different slopes were obtained in Period 1 of the study for the relationships between FM and dietary energy at different feeding events, the data were pooled in order to facilitate comparisons between sections. The pooled results for Period 1 were compared to the pooled results for Period 2. The prediction of FM from dietary energy was more precise in Period 1 than in Period 2.

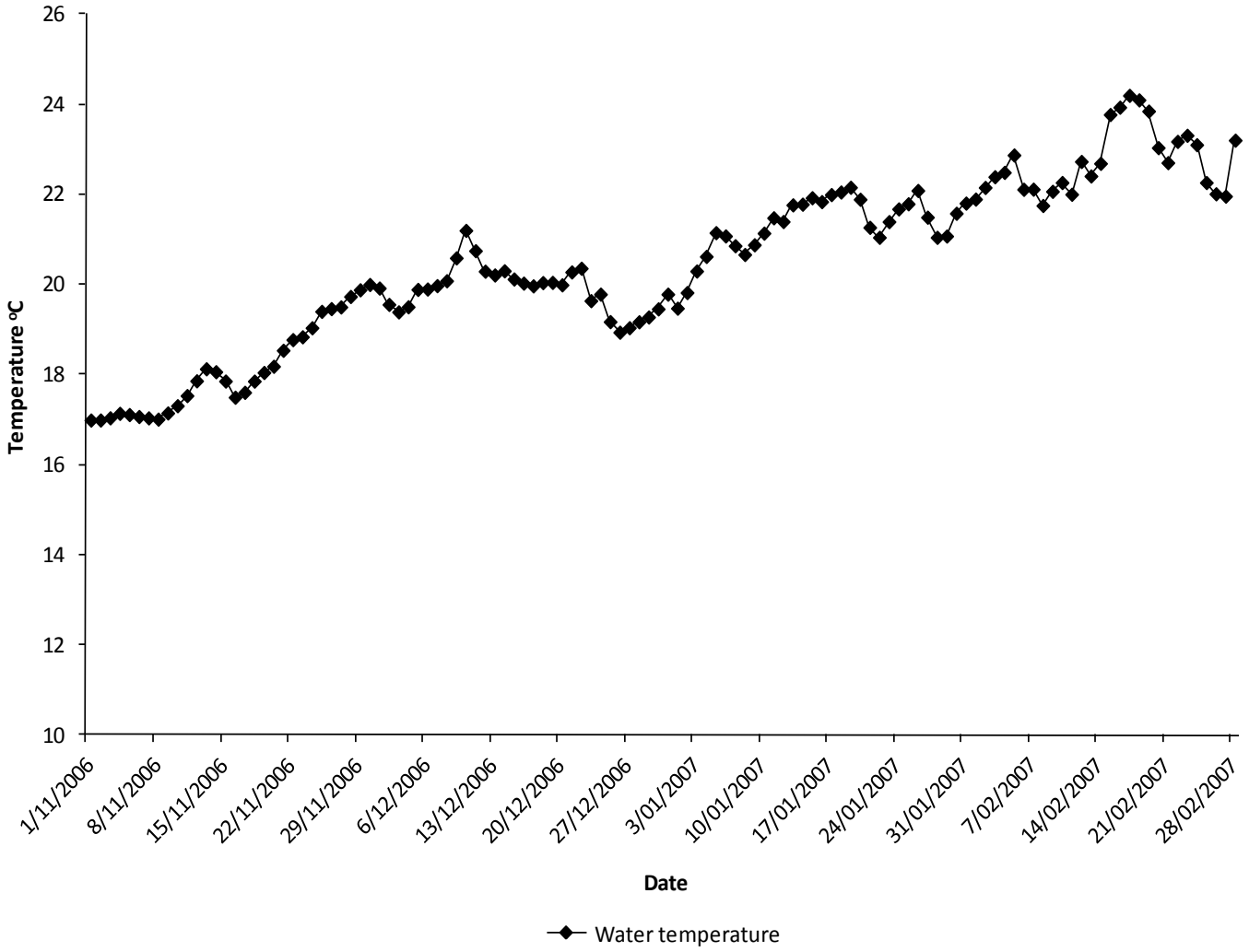


Figure 3.2.1 Mean daily water temperature (^{o}C) during the experimental Period (date) measured at a depth of 5m using a Vemco data recorder.

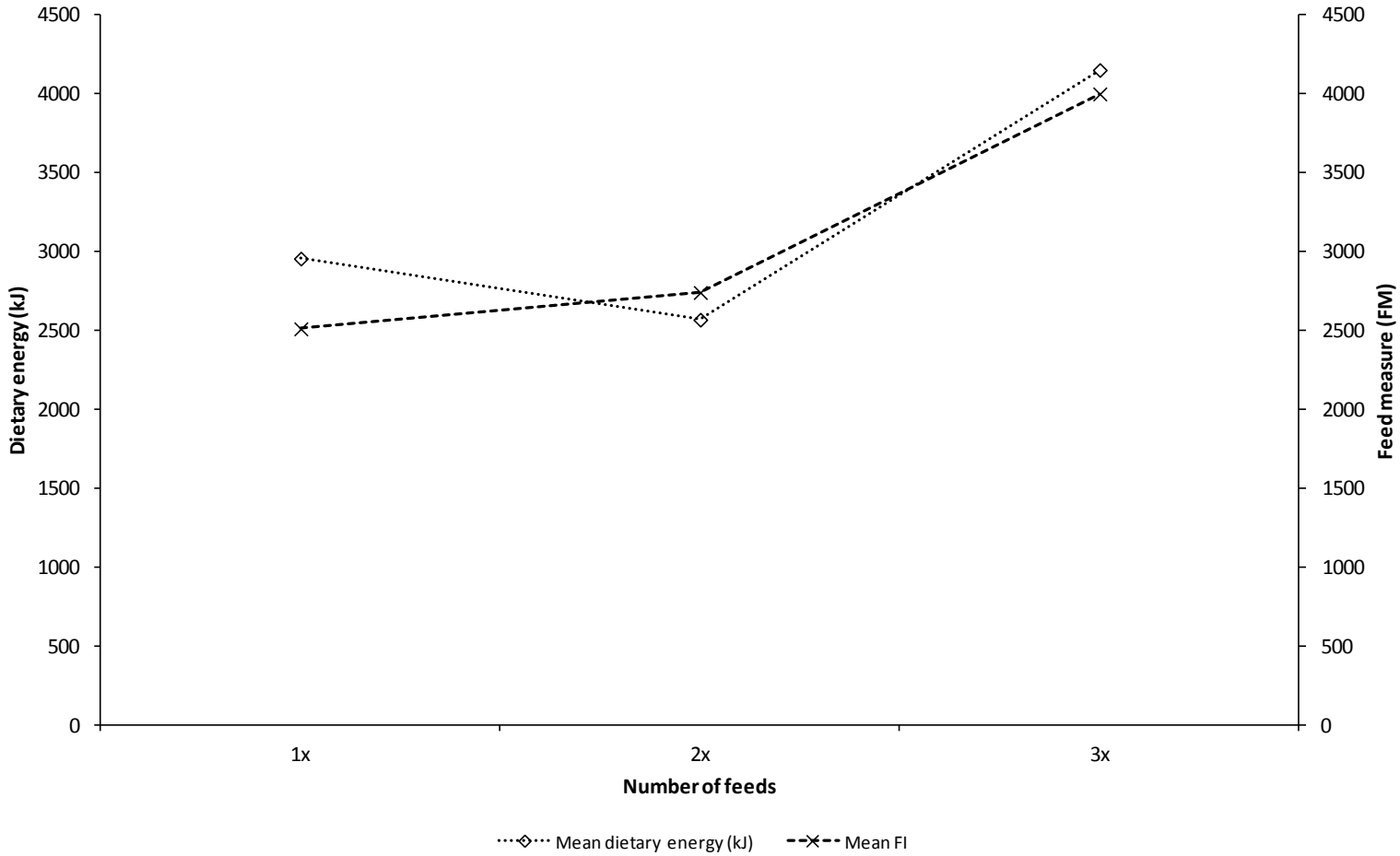


Figure 3.2.2 Trial 2 Period 1: The relationship of mean dietary energy (kJ) consumed plotted on the primary Y axis and mean feed measure (FM) plotted on the secondary Y axis in response to feeding regime.

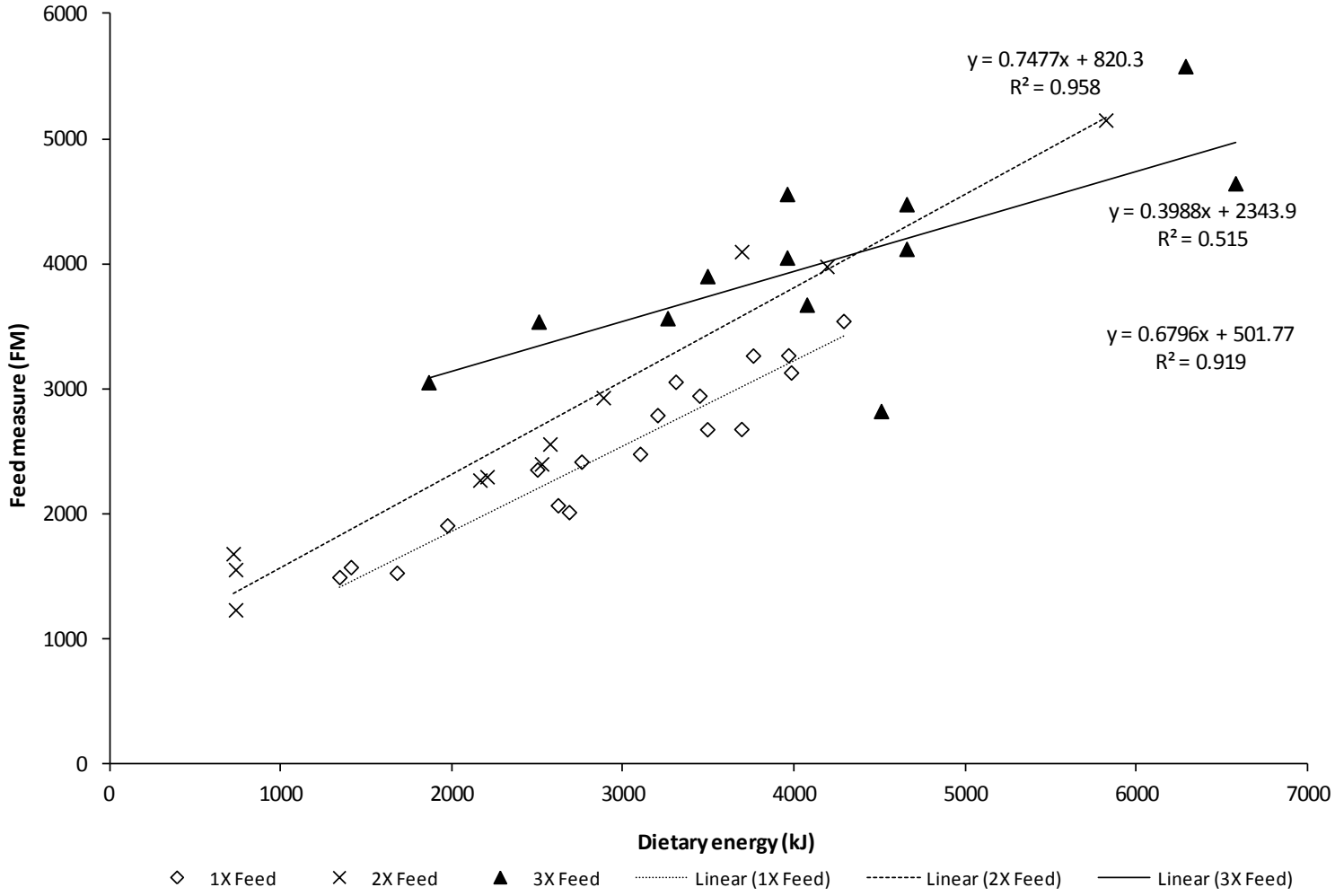


Figure 3.2.3 Trial 2 Period 1: General linear regression analysis of the relationship between feed measure (FM) and dietary energy (kJ) in response to feeding regime.

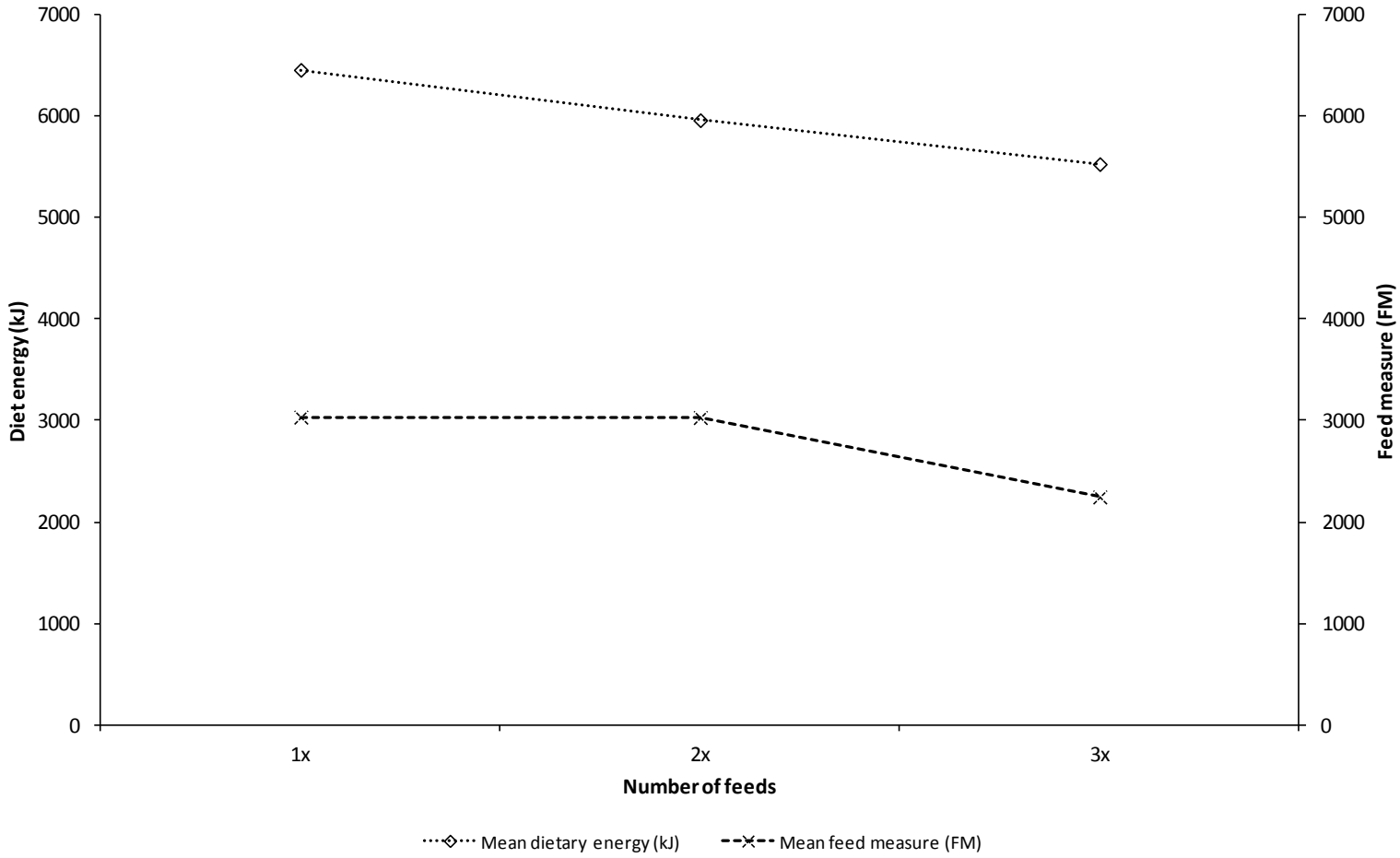


Figure 3.2.4 Trial 2 Period 2: The relationship of mean dietary energy (kJ) consumed plotted on the primary Y axis, p = and mean feed measure (FM) plotted on the secondary Y axis in response to feeding regime.

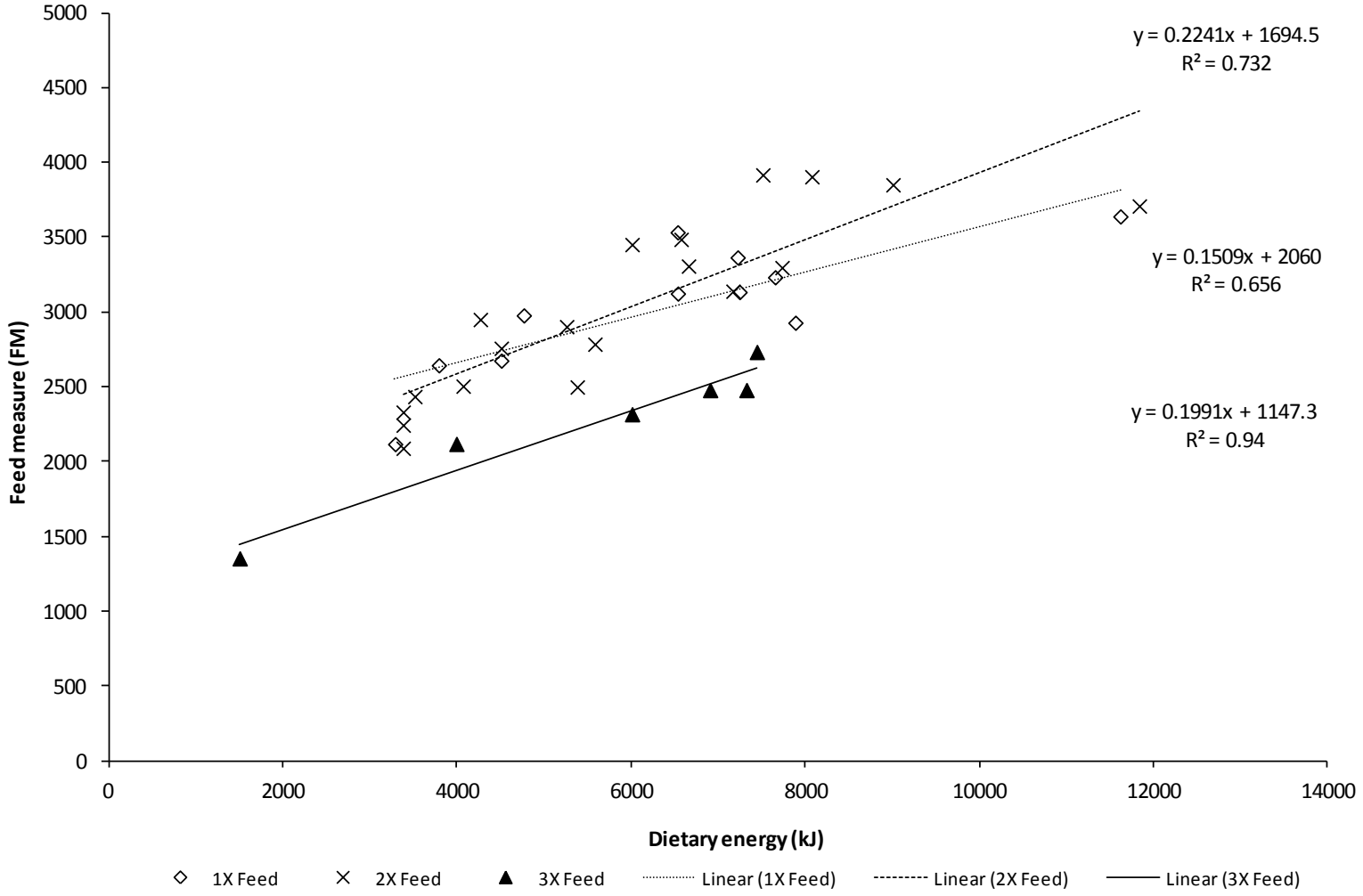


Figure 3.2.5 Trial 2 Period 2: General linear regression analysis of the relationship between feed measure (FM) and dietary energy (kJ) in response to feeding regime.

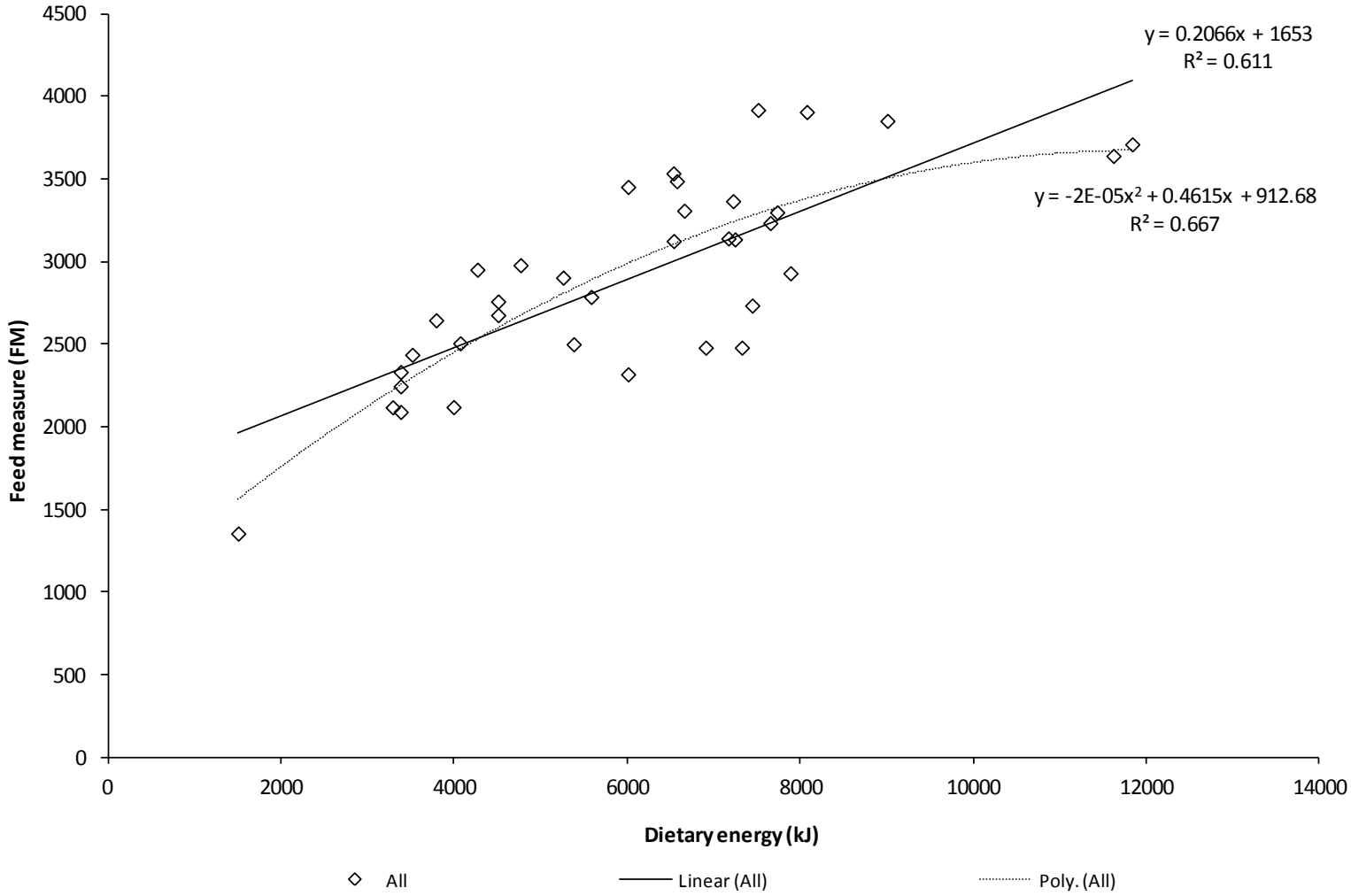


Figure 3.2.6 Trial 2 Period 2: General linear regression analysis of feed measure in response to dietary energy (kJ) for pooled data from all feeding regimes.

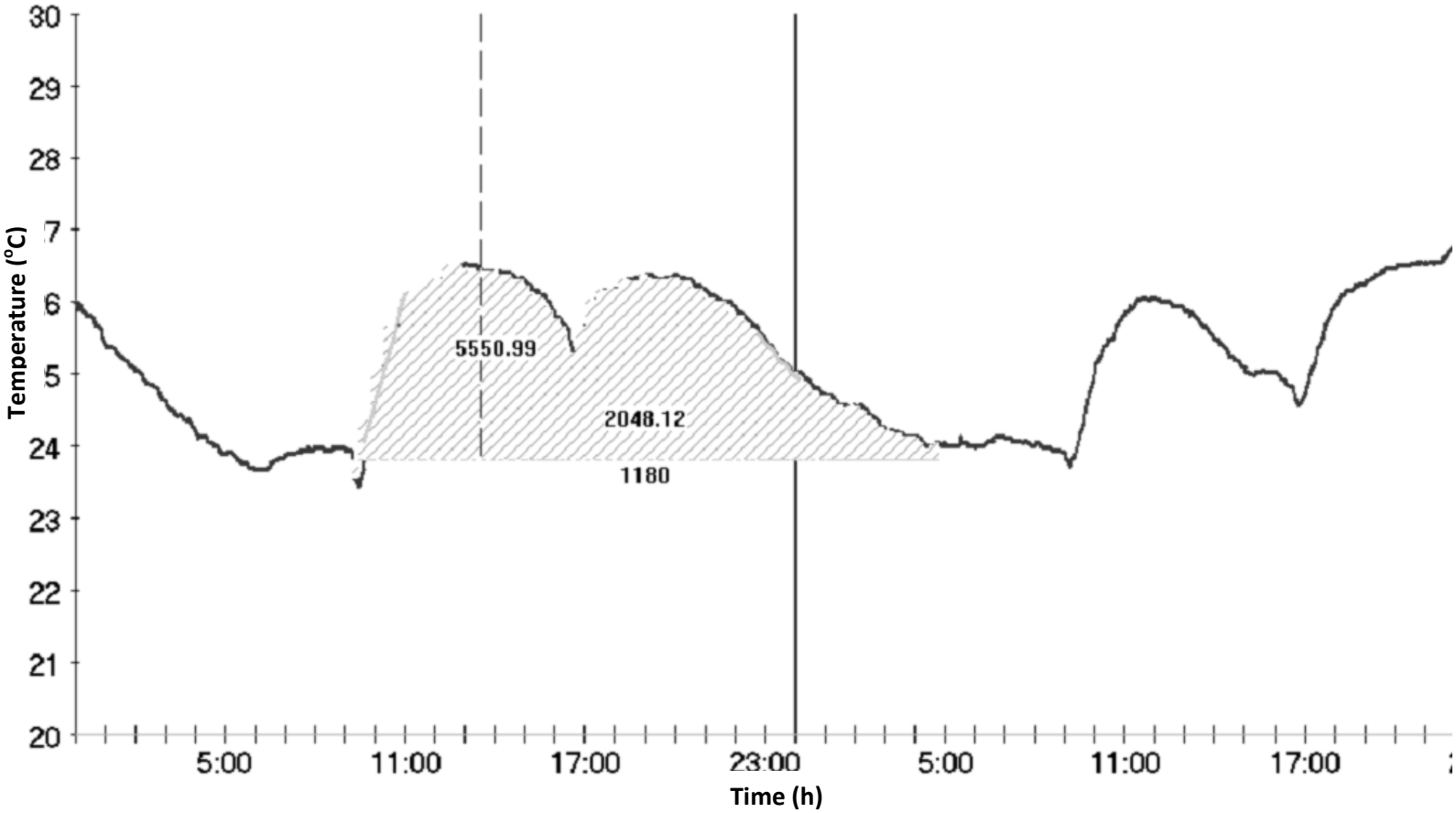


Figure 3.2.7 SBT visceral warming pattern expressed as a function of temperature area under the curve (FM) in relation to time (h) to two feeding events of the Australian sardine *S. sagax*.

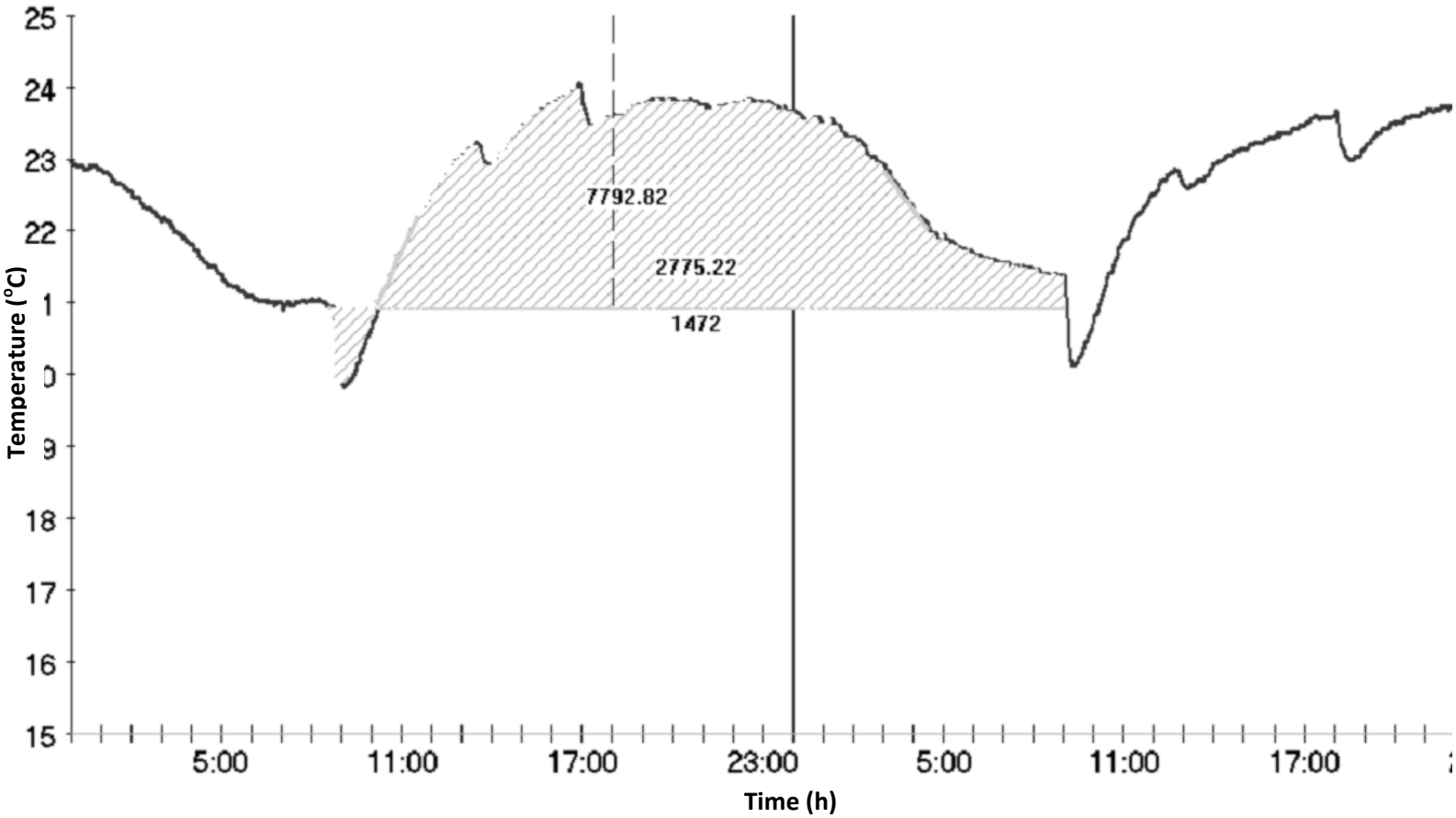


Figure 3.2.8 SBT visceral warming pattern expressed as a function of temperature area under the curve (FM) in relation to time (h) to three feeding events of the Australian sardine *S. sagax*.

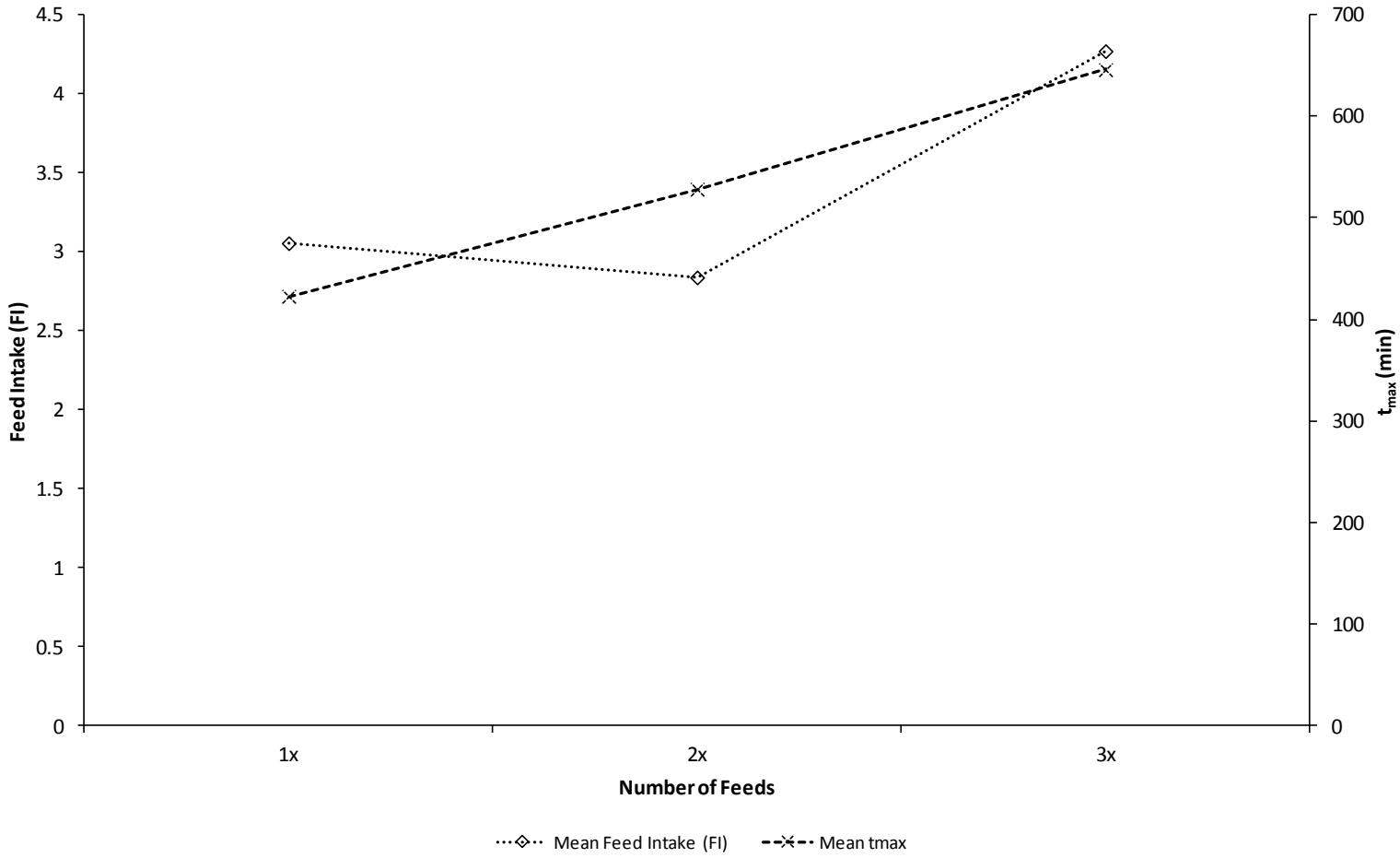


Figure 3.2.9 Trial 2 Period 1: The relationship of feed intake (FI) consumed expressed as % body weight per day plotted on the primary Y axis and t_{max} (min) plotted on the secondary Y axis in response to feeding regime.

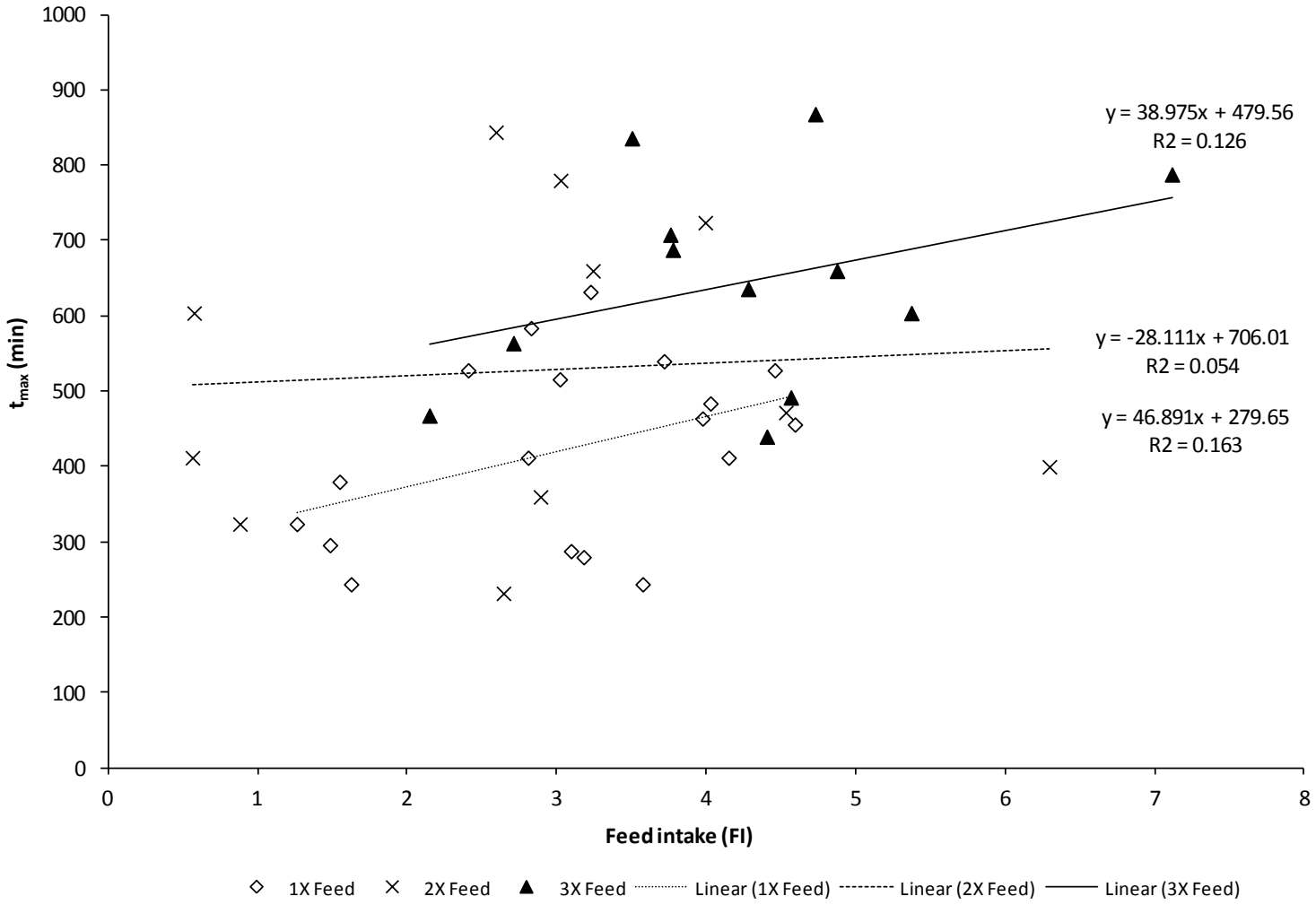


Figure 3.2.10 Trial 2 Period 1: General linear regression analysis of the relationship between t_{max} (min) and intake (FI) consumed expressed as % body weight per day and feeding regime.

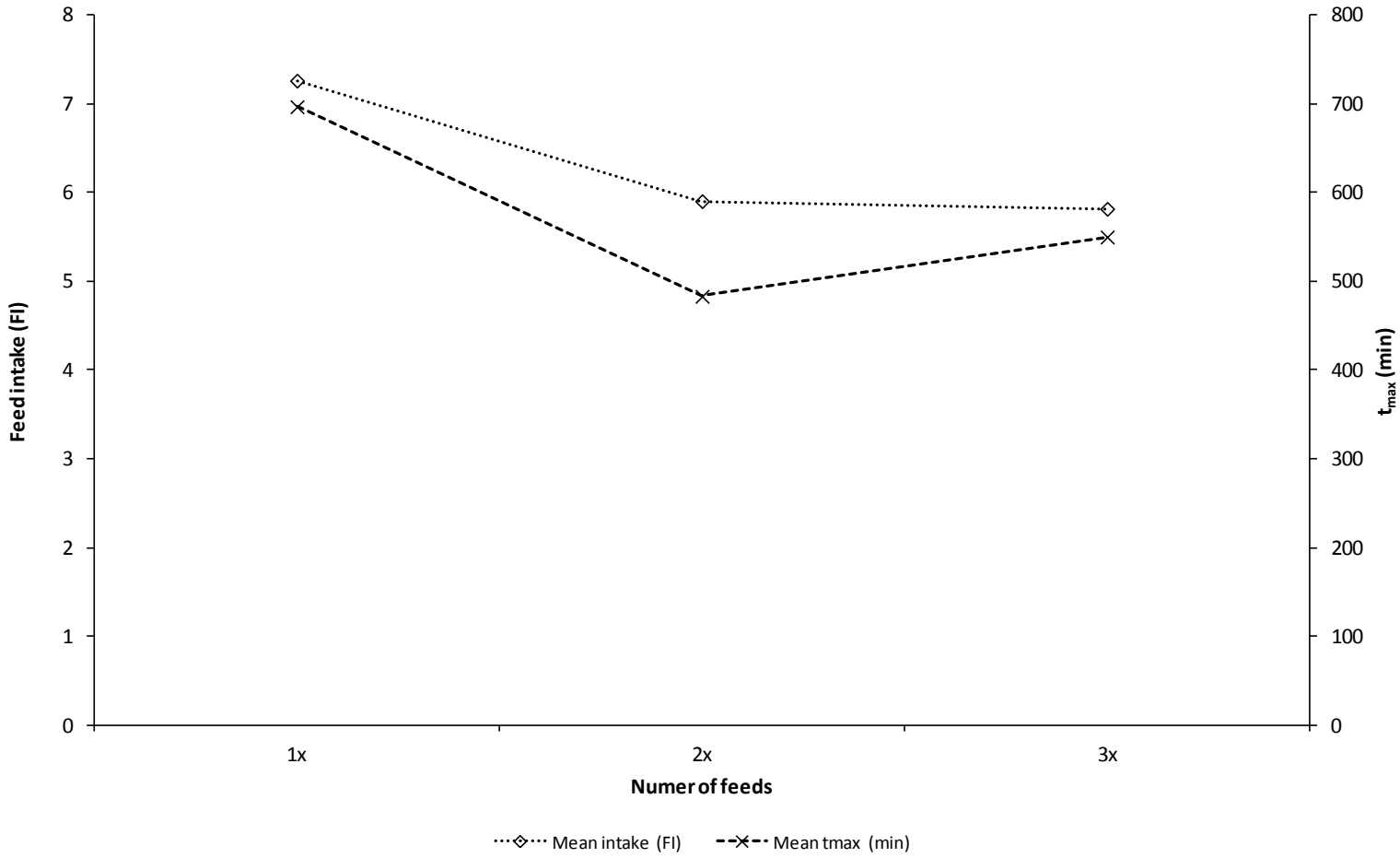


Figure 3.2.11 Trial 2 Period 2: The relationship of feed intake (FI) consumed expressed as % body weight per day plotted on the primary Y axis and t_{max} (min) plotted on the secondary Y axis in response to feeding regime.

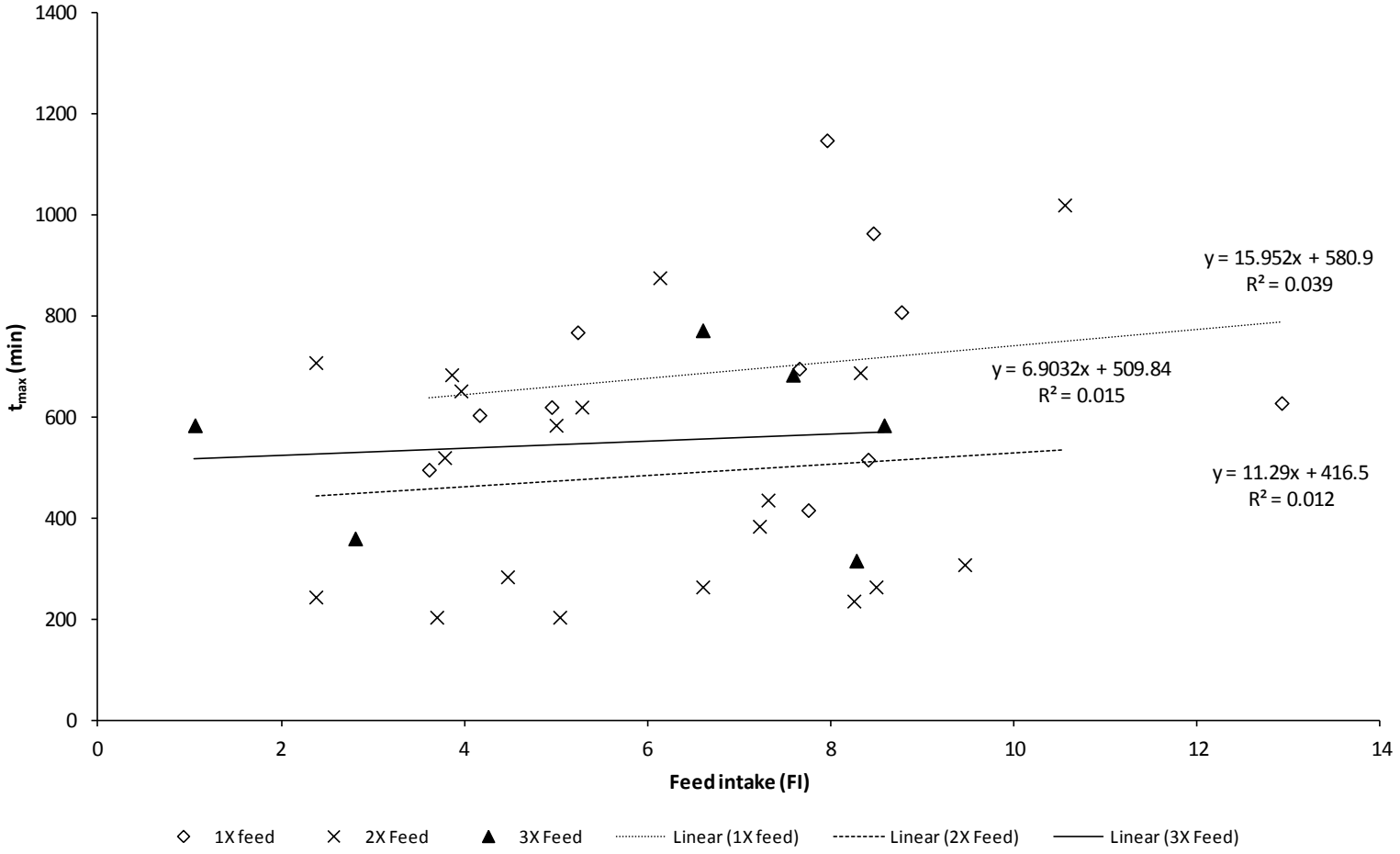


Figure 3.2.12 Trial 2 Period 2: General linear regression analysis of the relationship between t_{max} (min) and intake (FI) consumed expressed as % body weight per day and feeding regime.

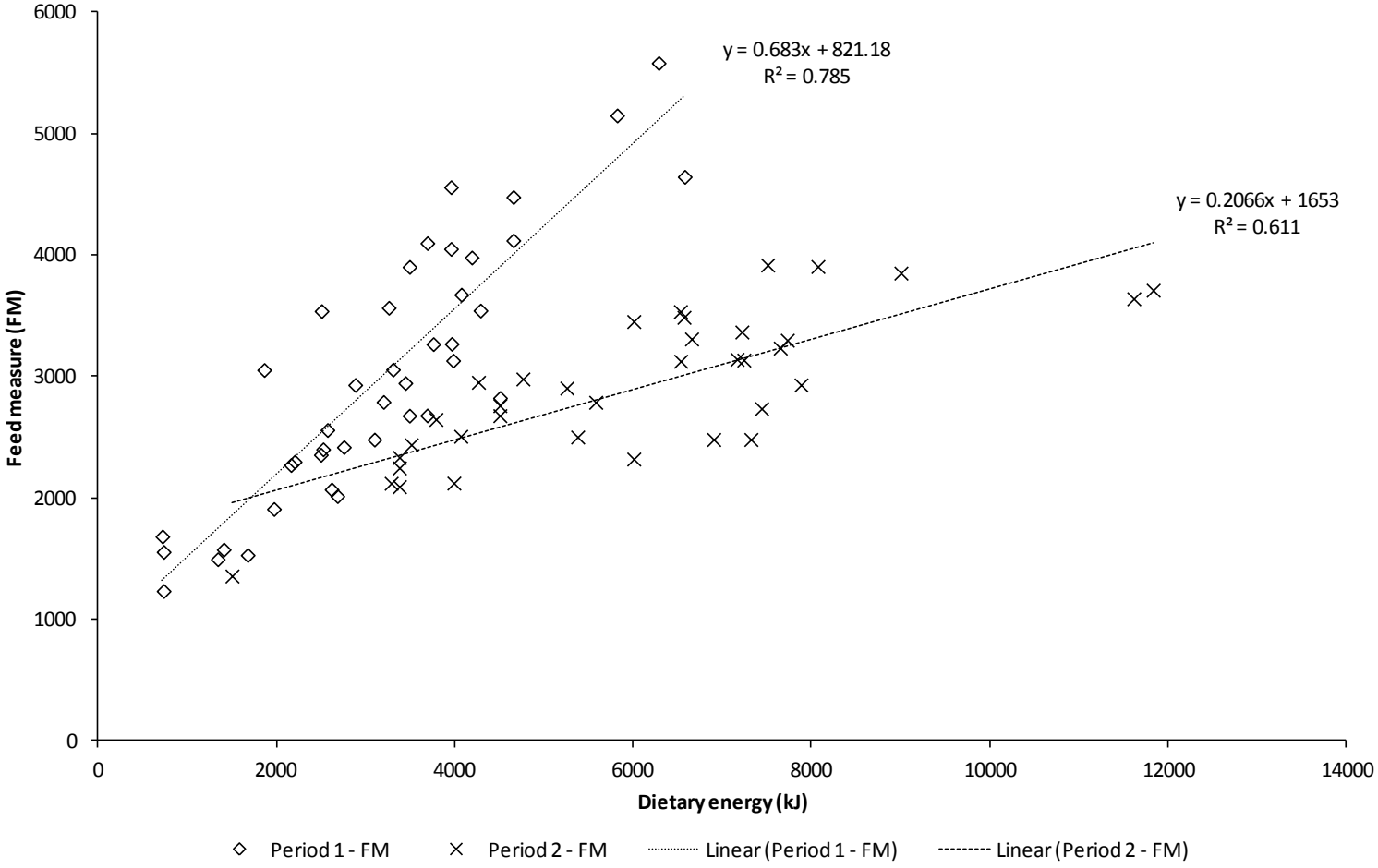


Figure 3.2.13 Trial 2 Period 2: General linear regression analysis of the relationship between feed measure (FM) in response to dietary energy (kJ).

Trial 1 and 2 Combined - the relationships between basal and maximum visceral temperatures and water temperature

The results from Trial 2 indicated further analysis of data was required to understand basal and maximum visceral temperature patterns in experimental fish. To this end, data from Trials 1 and 2 were analysed to determine relationships between basal and maximum visceral and ambient water temperatures.

Linear regression was used to determine the relationship between water temperature (T_w), basal temperature (T_b) and maximum visceral temperature (T_{max}). Significant, strong linear relationships were evident for both T_b (F = 3395.885, df = 1, 378, p < 0.001) and T_{max} (F = 1274.720, df = 1.378. p < 0.001). The resulting equations were as follows: T_b: 0.7723x + *7.4119* $R^2 = 0.9$; T_{max}: *0.6761x + 12.673* $R^2 = 0.771$ (Figure 3.2.14). The R^2 values were large indicating that a considerable proportion of the variability in T_b and T_{max} could be predicted by T_w.

Analyses were conducted to determine whether the slopes between T_b and T_{max} differed. This was conducted by transforming the data to long format, dummy-coding the temperature type, and running GLM analyses with an interaction. As would be expected, significant differences were observed for the intercept values between T_b and T_{max} (difference of 5.261; F = 156.738, df = 1,756, p < 0.001). However, there were also significant differences in the slope values. The slope of *0.7713x* for T_b was significantly higher than the slope of *0.6761x* for T_{max} (F = 17.332, df = 1, 756, p < 0.001). Therefore, T_b was more strongly predicted by T_w than was T_{max}.

Figure 3.2.15 shows a gap in the data points for T_w between about 20^{o}C and 22^{o}C, making it difficult to estimate the relationships in this range. In addition, there appears the possibility of a structural break in the relationships of T_{vis} to T_w when the T_w reaches approximately 22^{o}C. It appears that the slope of the lines may "flatten" with higher T_w. Illustration of this can be seen in Figure 3.2.15, in which the data were split according to low and high T_w and modelled separately. It can be seen that the slopes for the higher temperatures were smaller than those at the lower temperatures.

To determine whether these changes were statistically significant, analyses were conducted using GLM by creating a dummy coded variable for temperature (0 ≤20^{o}C, 1 ≥ 21^{o}C), i.e. by running a Chow test for equality of coefficients. Inclusion of the dummy variable in the model assesses the difference in intercepts for the two T_{vis} groups, and the dummy by temperature interaction assesses whether there was any difference in slopes.

For T_b, the difference in the intercept at low and high temperatures (14.391) was statistically significant (F = 33.262, df = 1, 376, p < 0.001), although this was perhaps to be expected given the vast temperature differences between the data points used for creating the model in the two groups. There was also a significant slope difference between the values at low and high T_w. The slope of *0.9308x* at lower T_w was significantly different from the slope of *0.2307x* at higher T_w (F = 40.045, df = 1, 376, p < 0.001). Therefore, T_b was predicted very strongly by T_w up to a T_w of approximately 19.9^{o}C. At water temperatures above 21.7^{o}C, there was a much smaller relationship between T_w and T_b. At exactly which T_w the structural break occurs cannot be deduced given the absence of data points in the break range.

For T_{max}, the difference between the intercepts at low and high T_w (5.064) was not statistically significant (F = 1.499, df = 1, 376, p = 0.222). Furthermore, the slope of *0.7232x* at low T_w and the slope of *0.4806x* at high T_w were not significantly different (F = 1.750, df = 1, 376, p = 0.187). Taken together, the specification of separate slopes and intercepts for the two T_w did not result in a significant increase in prediction of T_{max} (p = 0.092). Therefore, although a somewhat smaller slope was evident for T_{max} at higher T_w, the change in slopes was not significantly different. Therefore, based on these results a linear model across the range of T_w data points appears to be the most appropriate equation for modelling T_{max} in this dataset.

Summary

Whilst the slopes of the linear regression analysis differed the results showed that T_b was more strongly predicted by T_w than was T_{max}. There was a gap in data points for T_w between about 20 ^{o}C and 22^{o}C and a structural break in the relationships of T_{vis} to T_w was explored as it appeared that the slope of the lines may plateau with higher water temperatures.

There was a significant slope difference between the values at low and high T_w. Therefore, T_b was predicted very strongly by T_w up to a T_w of approximately 19.9^{o}C. At water temperatures above 21.7^{o}C, there was a much smaller relationship between T_w and T_b. At exactly which T_w the structural break occurs cannot be deduced given the absence of data points in the break range. However, based on these results a linear model across the range of T_w data points appears to be the most appropriate equation for modelling T_{max} in this dataset and further investigations need to be explored.

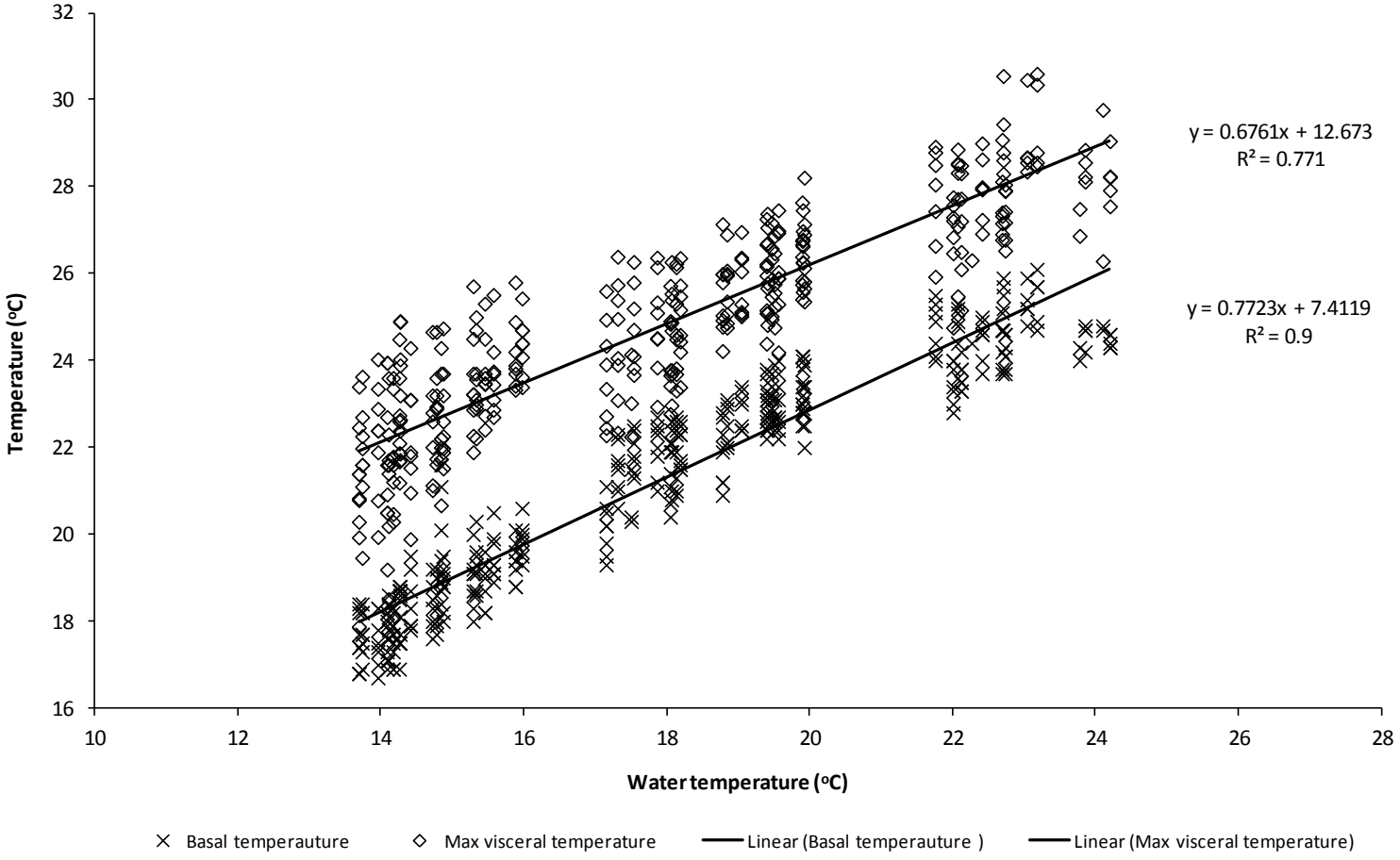


Figure 3.2.14 Basal and maximum visceral temperatures in relation to ambient water temperature.

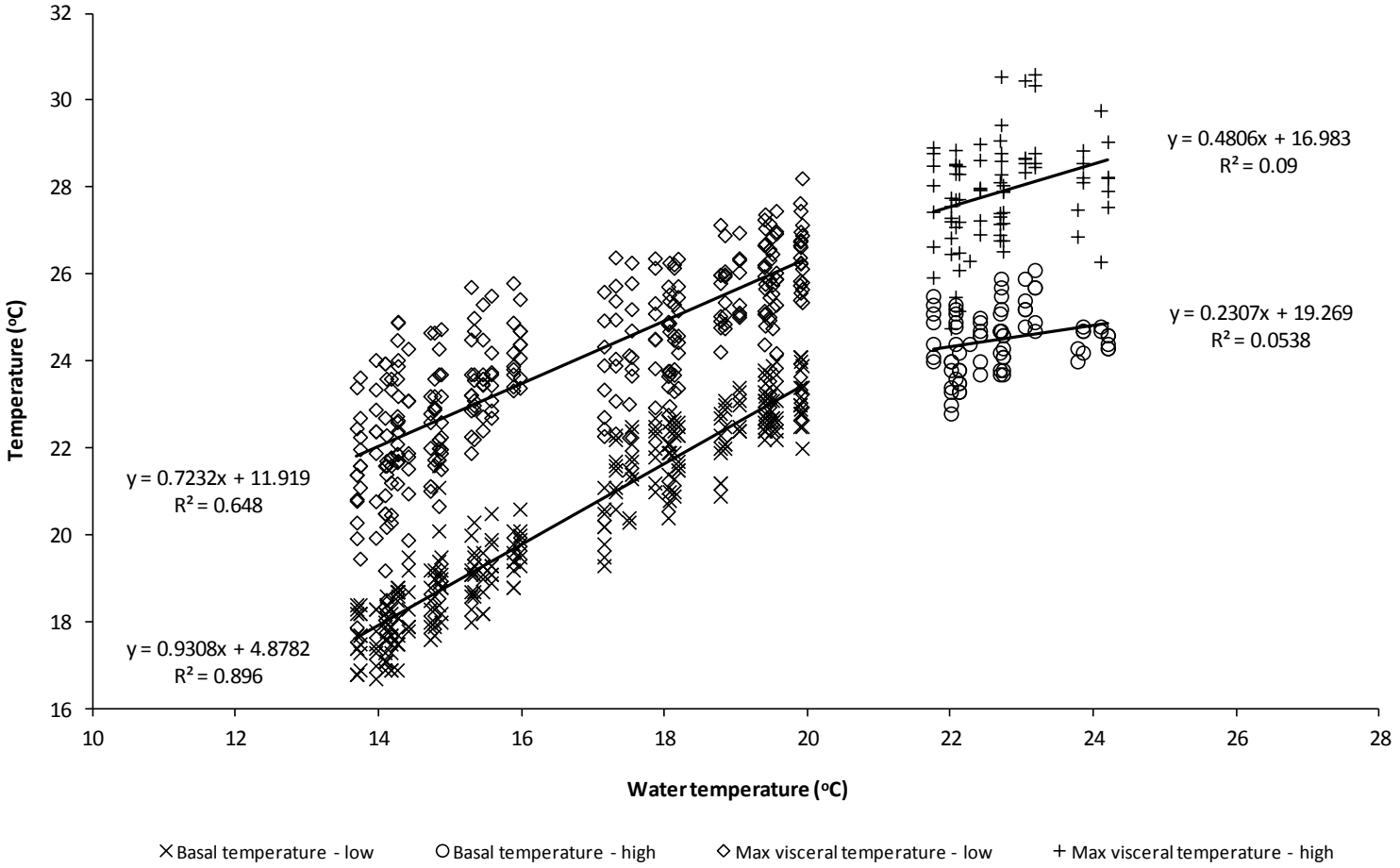


Figure 3.2.15 Basal and maximum visceral temperatures in relation to water temperature, with separate regression lines for low and high water temperatures. Assessing the possibility of a structural break in the relationships of T_{vis} to T_w when the T_w reaches approximately 22°C. Analyses were conducted using GLM by creating a dummy coded variable for temperature (0 ≤20°C, 1 ≥ 21°C), i.e. by running a Chow test for equality of coefficients.

3.3 Trial 3 – The measurement of temperature in red muscle, white muscle and the visceral cavity of slaughtered southern bluefin tuna (*Thunnus maccoyii*) in response to three feeding regimes and ambient water temperature

Trials 1 and 2 showed that visceral warming patterns are likely to be strongly influenced by dietary energy, feed frequency and water temperature. When basal and maximum visceral temperatures were plotted against ambient water temperature the combined results suggest that there are physiology control mechanisms to manage visceral heat. Trial 3 involved analysis of temperature profiles of red and white muscle and viscera in response to three feeding regimes and ambient water temperature to assess regional endothermy relationships.

This trial builds on previous research (Carey et al., 1984; Graham and Dickson, 2000; Sepulveda et al., 2007) by exploring regional endothermy and visceral warming in SBT, and the thermoregulation response of SBT in relation to water temperature and feeding (Graham and Dickson, 2000; Graham and Dickson, 2001; Dickson and Graham, 2004). The mean temperatures for each feeding regime are shown in Table 3.3.1 and Figure 3.3.1.

Separate General Linear Model (GLM) analyses were conducted to predict each body temperature from the treatment group and the water temperature (T_w).

For the red muscle temperature (T_{rm}), there was no effect of feeding treatment (F = 0.255, df = 2, 96, p = 0.775) or of T_w (F = 0.906, df = 1, 96, p = 0.344). As seen by the means (Table 3.3.1), the T_{rm} appears to be maintained at approximately 30^{o}C regardless of the T_w or feeding regime.

For the white muscle temperature (T_{wm}), there was a significant effect of T_w (F = 269.298, df = 1, 96, p < 0.001) but no effect of treatment (F = 1.520, df = 2,96, p = 0.224). With the treatments pooled, the resulting regression equation between T_{wm} and T_w was $Y = 1.0147x + 5.7037$, $R^2 = 0.7432$. The slope of nearly 1 indicates that T_{wm} was maintained at approximately 5.7^{o}C above T_w, and increases linearly in a degree for degree fashion, at least in the range of temperatures being studied here.

Table 3.3.1 Mean ±SE n=10 of red and white muscle and visceral core temperatures (^{o}C) taken during commercial harvests in response to three feeding treatments including the previous afternoon (12 h), previous morning (24 h) or 2 days (48 h) before harvest.

Feeding regime	Red muscle – (^{o}C)	White muscle – (^{o}C)	Visceral – (^{o}C)	Sea water – (^{o}C)
No feed 48 h	30.4 ±0.49	19.8 ±0.25	21.8 ±0.72	14.2
No feed 24 h	30.3 ±0.40	23.8 ±0.23	23.7 ±0.20	18.0
	30.8 ±0.32	20.2 ±0.26	20.5 ±0.29	14.2
	29.4 ±0.35	19.5 ±0.19	21.5 ±0.45	14.0
No feed 12 h	30.4 ±0.41	22.5 ±0.17	24.5 ±0.26	16.0
	29.8 ±0.50	22.1 ±0.41	22.6 ±0.42	16.0
	30.5 ±0.34	22.9 ±0.37	23.6 ±0.48	16.8
	30.1 ±0.33	20.6 ±0.17	22.1 ±0.45	14.5
	29.8 ±0.28	19.2 ±0.26	22.9 ±0.45	13.7
	30.2 ±0.49	19.7 ±0.25	23.1 ±0.46	13.4

The temperature of the visceral cavity (T_{vis}) was affected by both T_w (F = 19.706, df = 1, 96, p < 0.001) and feeding treatment (F = 9.230, df = 2, 96, p < 0.001). Analysis of the marginal means indicated that, with the T_w held constant, the "No feed 12 h" group had a higher mean T_{vis} than the other two feeding treatments.

The "No feed 48 h" group consisted of only one sample due to commercial constraints. Given the absence of variability in the T_w for this group, the relationship between the T_{vis} and T_w could not be appropriately modelled for this group. Thus, this sample was excluded from the analyses for the investigation of an interaction term.

Re-analysis using only the "No feed 12 h" and "No feed 24 h" groups with the inclusion of an interaction term in the GLM model revealed significant effects for each model term:

T_w (F = 23, 170, df = 1, 86, p < 0.001), treatment group (F = 6.745, df = 1, 86, p = 0.011), and the interaction between T_w and treatment (F = 4.570, df = 1, 86, p = 0.035). This indicates that the two feeding treatments had different relationships between T_w and T_{vis}. As seen in Figure 3.3.2, the relationship between T_{vis} and T_w for the "No Feed 12 h" group was best expressed as: $Y = 0.2613x + 19.23$, $R^2 = 0.05$. This was almost a flat, non-existent relationship. For the "No Feed 24 h" group, the linear expression was $Y = 0.6788x + 11.468$, $R^2 = 0.565$. This indicates that the T_w was a much better predictor of T_{vis} when the SBT had gone without feed for a longer period of time.

Comparison of body temperatures to water temperature

Based on the previous analyses, it appeared that the three measured body temperatures had different relationships with T_w. This was assessed formally using GLM. For this analysis, the T_{vis} were pooled across all three feeding regimes. The results indicated a significant effect of T_w (F = 98.461, df = 1, 294, p < 0.001), placement of temperature recording (F = 74.877, df = 2, 294, p < 0.001), and a significant interaction between T_w and the body site (F = 28.122, df = 2, 294, p < 0.001) with regard to the resultant body temperature recordings. Analysis of the parameter estimates indicated significant differences between the intercepts and the slopes of all three measurements.

The comparison was facilitated by analysis of results shown in Figure 3.3.3. T_{rm} had an intercept of nearly 30^{o}C and essentially no slope, indicating that it remained constant across T_w. Given that the slope parameter was non-significant, it was more prudent to use the mean T_{rm} rather than any adjustment based on T_w. The mean T_{rm} across all measurements was 30.2^{o}C. T_{wm} had an intercept of about 6^{o}C (5.7037) and a slope of approximately 1 (1.0147), indicating that T_{wm} was regulated at about six degrees above T_w. The T_{vis} had an intercept of 15.862, and a slope of 0.4503x (R^2 = 0.14). Thus, a weak relationship was observed between increasing T_w and increasing T_{vis}. However, this pooled visceral cavity analysis was somewhat misleading, since it was shown in the previous analysis that the relationship between T_{vis} and T_w was dependent on the feeding treatment.

Summary

Separate General Linear Model (GLM) analyses were conducted to predict each body temperature from the treatment group and water temperature. For the red muscle temperature there was no effect of feeding treatment and appears to be maintained at approximately 30^{o}C regardless of the T_w or feeding regime.

For the white muscle temperature , there was a significant effect of T_w but no effect of different feeding regime and suggests that T_{wm} is maintained at approximately 6^{o}C above T_w, and increases linearly in a degree for degree fashion, at least in the range of temperatures considered in this Trial.

The temperature of the visceral cavity was affected by both T_w and feeding as already shown in Trials 1 and 2. The results of this Trial showed that T_w was a much better predictor of T_b when the SBT had gone without feed for a longer period of time suggesting it takes longer for basal temperatures to be reached over the temperature range of observation.

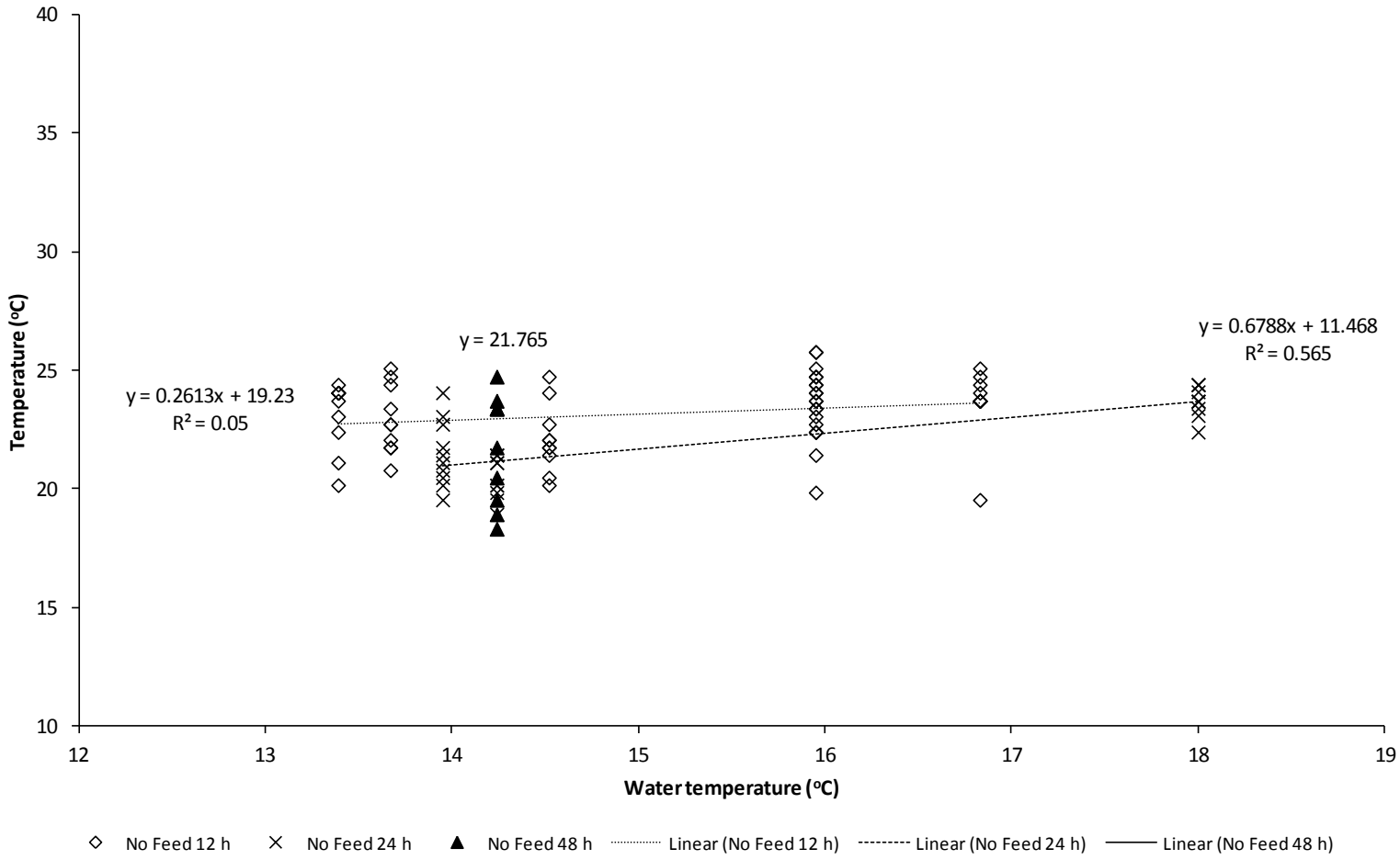


Figure 3.3.1 General linear regression analysis of visceral temperature (^{o}C) in relation to water temperature for each feeding regime. For the *No Feed 48 hours* group, only the point estimate (mean) is shown.

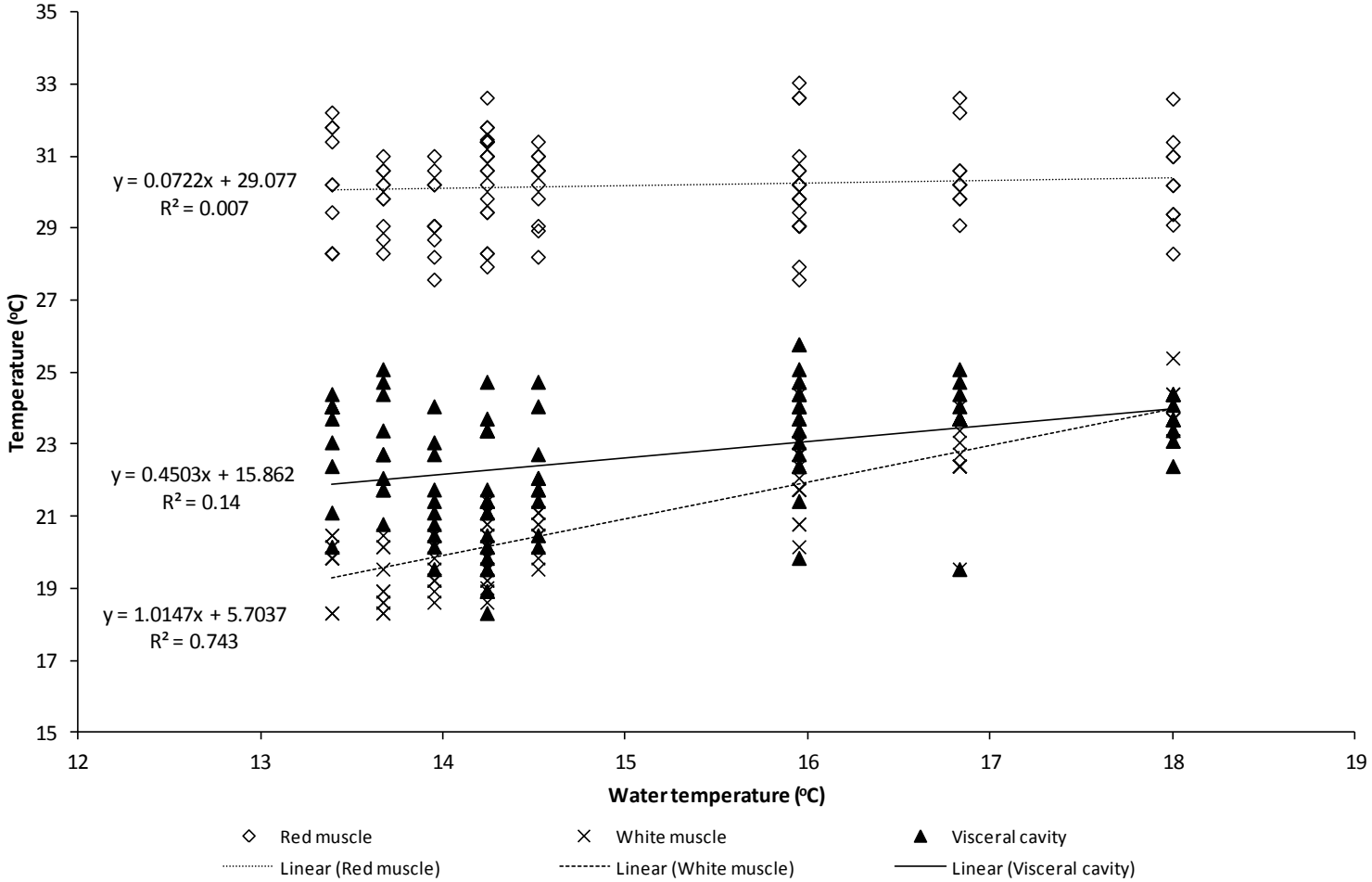


Figure 3.3.2 General linear regression analysis of red muscle, white muscle, and visceral cavity temperatures (^{o}C) on water temperature, pooled across feeding treatments.

3.4 Trial 4 – The measurement of maximum and basal visceral temperature in commercially cultured southern bluefin tuna (*Thunnus maccoyii*) in response to commercial feeding practices at ambient water temperatures

Results of Trials 1, 2 and 3 indicated that visceral warming patterns are affected by dietary energy and water temperature. It is well accepted that regional endothermy in tuna is strongly influenced by ambient water temperature (Graham and Dickson, 2001) and the results of Trial 3 are consistent with this.

CSIRO archival tag data enabled detailed exploration of SBT physiological responses to commercial feeding practices at different T_w as opposed to experimental manipulation in designed Trials. Comparisons between archival tag data and Trial data allowed for testing of visceral warming responses, physiology responses to feeding, and T_w. This Trial investigated the relationship between basal visceral temperature (T_b) and maximum visceral temperature (T_{max}) in relation to ambient water temperature (T_w) in commercially cultured SBT. Commercial data and experimental Trial data were compared to determine differences in T_b or T_{max} and whether tagging had an influence on physiology response of SBT.

Data from three tags out of 12 were excluded from the analysis as the T_w values significantly varied from known T_w values recorded in the tuna farming zone for the same period. In another two tags, the T_b was lower than the recorded T_w at the same point in time. Given current information on tuna physiology, these results were considered erroneous and were excluded from the analysis.

The mean temperatures for each sample at the available measurement sites are shown in Table 3.4.1. Table 3.4.1 has missing data where samples were not taken in Trials. Specifically, Trial 3 data was obtained from SBT that did not have implanted archival tags, whilst archival tags provided data in Trials 1, 2 and 4. Comparison of the means between CSIRO archival tags and the Trial 1 and 2 data showed that T_b for the CSIRO data were about 2^oC higher than the research data ($F = 167.047$, $df = 1, 1200$, $p < 0.001$), although the corresponding T_w were also about 2^oC higher ($F = 111.254$, $df = 1, 1200$, $p < 0.001$). The mean T_w for Trial 3 was the lowest of the three samples, although T_b was not recorded in this Trial.

Table 3.4.1 Mean results (±SE) of temperature measurement sites (^{o}C) for commercial and research samples used in this study.

Trial	Basal – (^{o}C)	Max visceral – (^{o}C)	White muscle- (^{o}C)	Red muscle – (^{o}C)	Water – (^{o}C)
Trial 4 *(n = 822)*	23.4 ±0.09	27.0 ±0.08	--	--	19.7 ±0.09
Trials 1 and 2 *(n = 380)*	21.3 ±0.13	24.8 ±0.12	--	--	18.0 ±0.16
Trial 3 *(n = 100)*			21.0 ±0.17	30.2 ±0.13	15.1 ±0.15

Comparison of basal and maximum visceral temperature in response to water temperature for CSIRO data

Separate GLM analyses were initially conducted to predict each body temperature from T_w. For T_b, the prediction from T_w was statistically significant (F = 2623.366, df = 1,820, p < 0.001). The regression equation used was $y = 0.8923x + 5.7465$, $R^2 = 0.762$ (Figure 3.4.1). The prediction of T_{max} was also significant (F = 1921.983, df = 1,920, p < 0.001), with a regression equation of $y = 0.7664x + 11.907$, $R^2 = 0.701$. In other words, the rate increase for 1^{o}C of T_w was *0.8923x* for the T_b and *0.7664x* for T_{max}.

Analyses were conducted to determine whether the slopes between the T_b and T_{max} differed. This was conducted by transforming the data to long format, dummy-coding the temperature type, and running GLM analyses with an interaction. As would be expected, significant differences were observed for the intercept values between T_b and T_{max} (basal = 5.7465, maximum = 11.907, difference of 6.161; F = 157.253, df = 1640, p < 0.001). There were also significant differences in the slope values.

The slope of *0.8923x* for T_b was significantly higher than the slope of *0.7664x* for T_{max} (difference of 0.1259, F = 26.031, df = 1,1640, p < 0.001). Therefore, the relationship between T_w and T_b was closer to one than the relationship between T_w and T_{max}. These regression lines are shown in Figure 3.4.1. Despite the significant differences, the lines did appear to be parallel. Furthermore, it should be noted that these results are very similar to the differences between T_b and T_{max} in response to T_w seen in the analyses of the Trial 1 and 2 research data.

Evaluation of structural breaks in CSIRO data

A structural break in the T_b to T_w relationship was found in Trial 2. Although the scatter plots between T_b and T_{max} and T_w appeared linear (Figure 3.3.1), the possibility of structural breaks in the data was also investigated. The research data had a gap between 19.2^oC and 21.8^oC. To replicate this analysis in the CSIRO data, the temperature split was also created at 21.8^oC (although this was an arbitrary split point as there was no similar gap in the temperature records for the CSIRO data).

For T_b, there was a statistically significant difference in the intercept at low and high temperatures (difference of 15.345, F = 30.026, df = 1, 818, p < 0.001), although this was expected given the vast temperature differences between the data points used for creating the model in the two groups. There was also a significant slope difference between the values at low and high temperatures. The slope of *0.9607x* at lower temperatures was significantly different from the slope of *0.2797x* at temperatures above 21.8^oC (F = 31.146, df = 1, 818, p < 0.001). The R^2 for the model specifying separate intercepts and slopes at the two temperatures was 0.771. Compared to the original equation R^2 (0.762), the R^2 difference of 0.009 was statistically significant (p < 0.001). As with the research data, the strength of the association between T_b and T_w was less direct at high temperatures. The differences in the regression lines according to temperature are seen in Figure 3.4.2.

The analysis of the T_{max} also showed differences in slopes at low and high temperatures. There was a statistically significant intercept difference (difference = 13.997, F = 24.872, df = 1,818, p < 0.001) and slope difference (difference = 0.6321, F = 26.719, df = 1, 818, p < 0.001).

The R^2 associated with specifying separate intercepts and slopes for the high temperature data went from 0.701 to 0.714. The change of 0.013 was statistically significant ($p < 0.001$). The results show that, similar to the pattern seen with T_b, the slope between T_{max} and T_w above 21.8°C was less direct than the relationship at lower temperatures (Figure 3.4.2).

It should be noted that the change in R^2 values were relatively small for each analysis corresponding to about 1% of the variance in recorded temperature. Therefore, although statistically significant due in part to the large sample size, the practical significance of separate model specifications may be small but biologically important.

Comparison of basal to water temperatures for commercial and research data

This analysis determined whether the change in T_b according to T_w differed in the commercial and research samples. For this comparison, the linear relationships across the range of T_w values were used and the issue of structural breaks was ignored for the current analysis. The linear relationship in the CSIRO data between T_b and T_w was: $y = 0.8923x + 5.7465$, $R^2 = 0.762$. In the research data, the relationship was: $y = 0.7723x + 7.4119$ $R^2 = 0.9$. The differences were significant for the intercept (difference = 1.665, F = 12.616, df = 1, 1198, $p < 0.001$) and for the slope (difference = 0.120, F = 23.363, df = 1, 1198, $p < 0.001$). The R^2 value for a regression model pooled across samples was $R^2 = 0.815$. For the model with separate specification for research and commercial samples the R^2 was 0.827. The change in R^2 value of 0.012 was statistically significant ($p < 0.001$).

Summary of temperature parameters

The T_{vis} to T_w linear regression parameters established in this study is shown in Table 3.4.2. Where significant differences occurred between groups these are shown as separate bulleted subheadings.

Table 3.4.2 Linear regression parameters for each temperature measurement site, in relation to water temperature.

Temperature (^{o}C)	Equation	R^2 value
Red muscle (research)	0.0722x + 29.077	0.007
White muscle (research)	1.0147x + 5.7037	0.743
Basal temperature	0.8714x + 5.9939	0.815
- **Research basal**	0.7723x + 7.4119	0.9
○ **Water < 20^{o}C**	0.9308x + 4.8782	0.896
○ **Water > 21.8^{o}C**	0.2307x + 19.269	0.054
Max visceral temperature (research)	0.6761x + 12.673	0.771
○ **Water < 21.8^{o}C**	0.7232x + 11.919	0.648
○ **Water > 21.8^{o}C**	0.4806x + 16.983	0.09
- **Commercial basal**	0.8923x + 5.7465	0.762
○ **Water < 21.8^{o}C**	0.9607x + 4.5062	0.654
○ **Water > 21.8^{o}C**	0.2797x + 19.852	0.03
Max visceral temperature (commercial)	0.7664x + 11.907	0.701
○ **Water < 21.8^{o}C**	0.8618x + 10.188	0.594
○ **Water > 21.8^{o}C**	0.2297x + 24.185	0.023

From Trial 3, the T_{rm} was maintained at a constant of about 30^{o}C and the T_{wm} was recorded at about 6^{o}C above T_w. In lower T_w (i.e. 20^{o}C or cooler), the predicted T_b varied from about 3.5^{o}C to 4.5^{o}C above T_w for both research and commercial samples. Weak correlation between T_b and T_w was observed in the higher T_w. Although less direct of a relationship, the predicted T_{max} were between 7 ^{o}C and 10^{o}C above T_w for cooler T_w. The prediction of T_{max} from T_w at values above 21.8^{o}C was weak.

Summary

Comparison of the mean temperature values between CSIRO archival tags and Trial 1 and 2 data showed that T_b for the CSIRO data were about 2^oC higher than the research data reinforcing the findings of Trial 3 that sufficient time is required for basal temperature to be reached.

As was expected, significant differences were observed for the intercept values between T_b and T_{max} although the linear regression lines appear to be relatively parallel and are very similar to the differences between T_b and T_{max} in response to T_w seen in the analyses of the Trial 1 and 2 data. A structural break in the T_b to T_w relationship was explored using a temperature split created at the same point as in Trial 1 and 2 data.

For T_b, there was a statistically significant difference in the intercept at low and high temperatures, although this was expected given the vast temperature differences between the data used for creating the model in the two groups. There was also a significant slope difference between the values at low and high temperatures. As with the research data, the strength of the association between T_b and T_w was less direct at high temperatures. The analysis on the T_{max} also showed differences in slopes at low and high temperatures. The results show that, similar to the pattern seen with T_b, the slope between T_{max} and T_w above 21.8^oC was less direct than the relationship at lower temperatures.

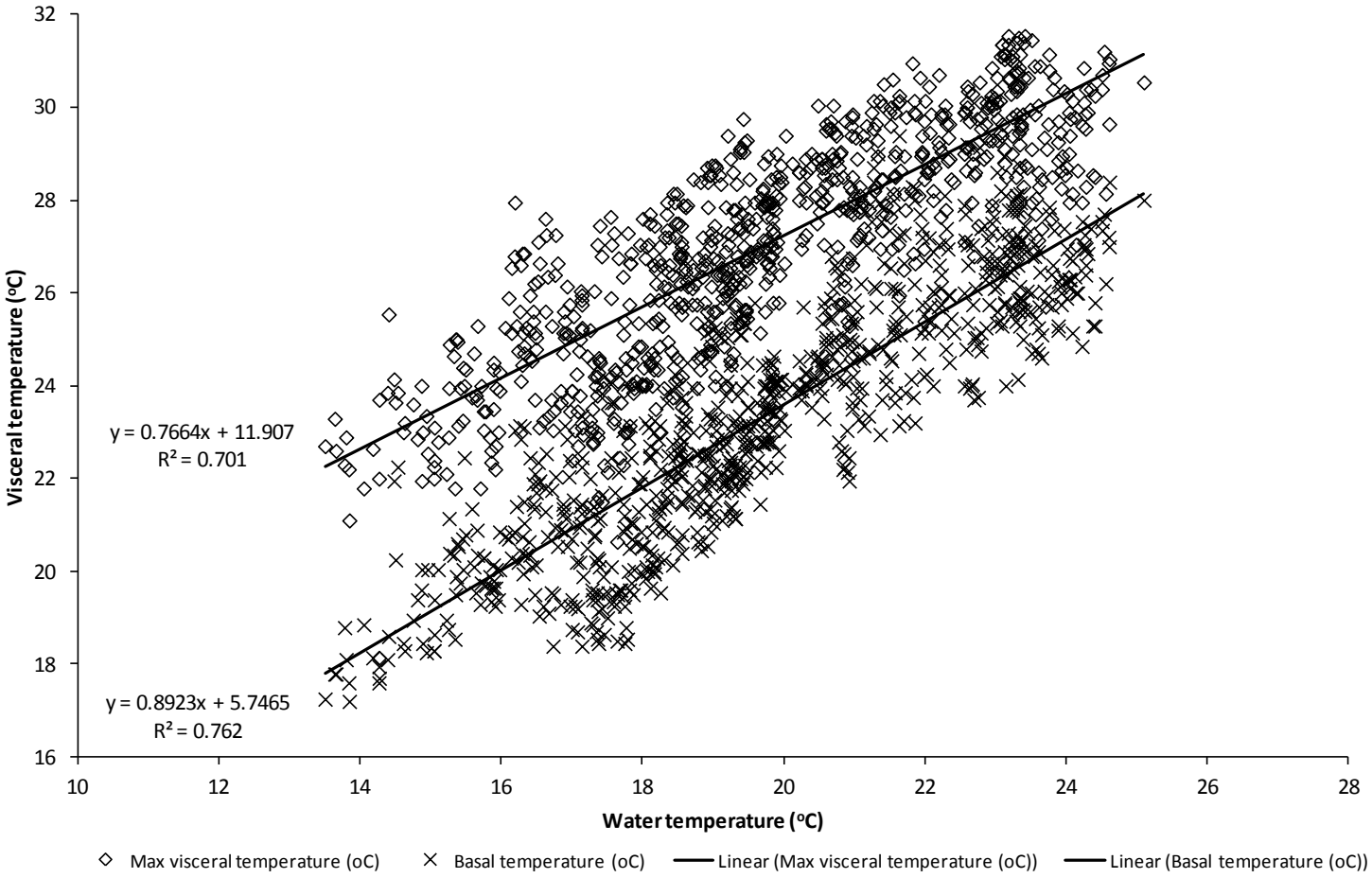


Figure 3.4.1 General linear regression analysis of basal and maximum visceral temperature (^{o}C) compared in response to water temperature (^{o}C) for CSIRO archival tag data.

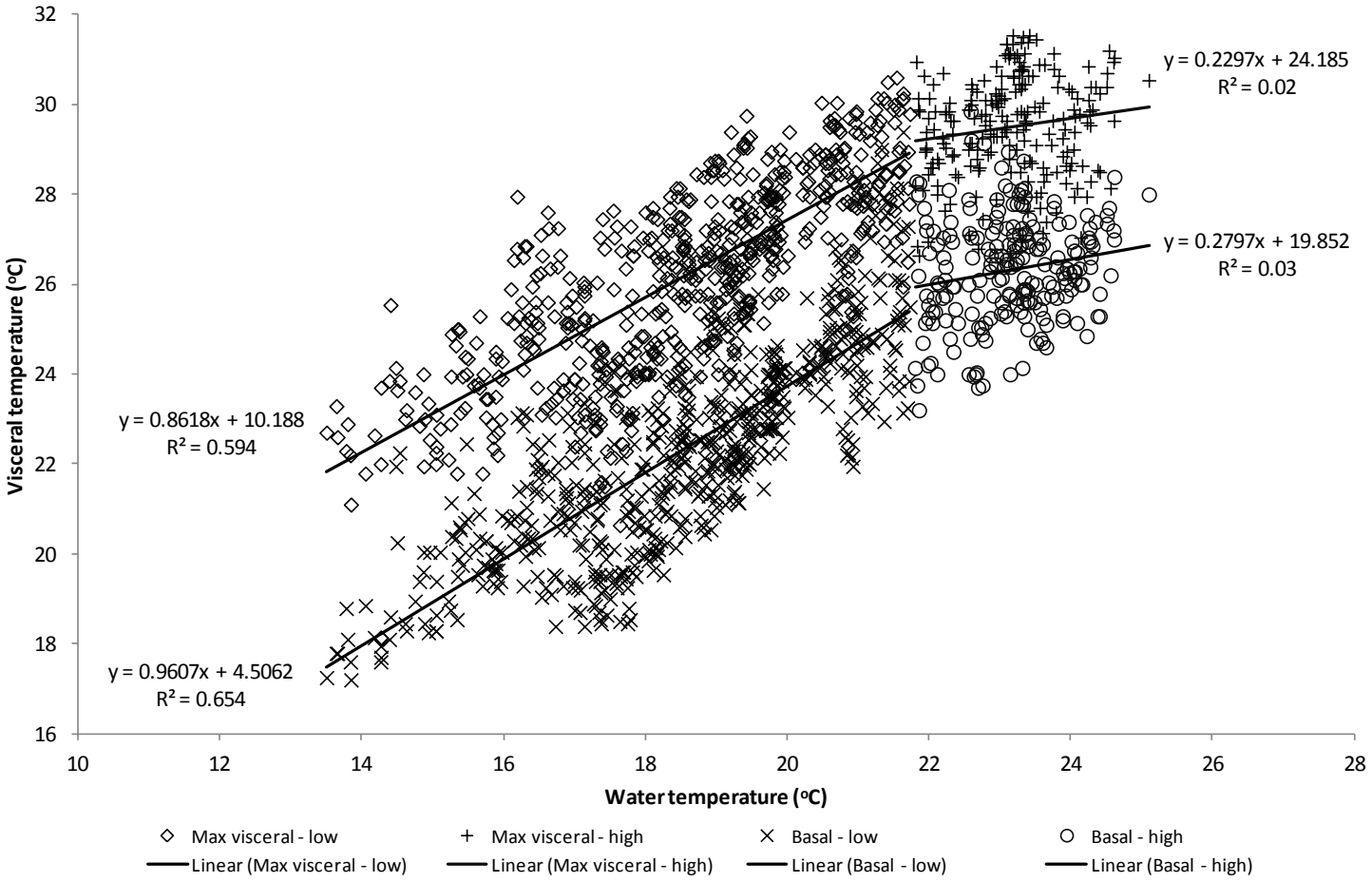


Figure 3.4.2 General linear regression analysis of basal and maximum visceral temperature (^{o}C) compared to water temperature (^{o}C) for CSIRO archival data with structural break between low and high water temperature.

3.5 Trial 5 – Feed intake, FCR, growth and proximate composition of southern bluefin tuna (*Thunnus maccoyii*) fed four diets in three periods over an 18 week period: *Can feed intake from visceral warming patterns be predicted?*

General linear models were developed in other Trials in this study to characterise visceral warming patterns as measures to predict feed intake. Whilst t_{max} has been discounted as a reliable measure due to feeding behaviour and the cumulative effect of meals on visceral warming, the results suggest that feed measure is a suitable model to predict energy intake providing dietary energy is known.

The *FORMU-BAIT*© feed optimisation software (van Barneveld and Ellis, 2007; van Barneveld et al., 2009) was used in Trial 5 to formulate combinations of baitfish diets varying in protein and lipid content to optimise SBT growth and feed intake over the farming season. Analyses explored the response of treatment (feeding regimes) on intake, growth, Food Conversion Ratio (FCR), Specific Growth Rate (SGR) and the nutrient proximate composition of SBT. A general linear model was applied to feed measure values to investigate the relationship between visceral warming patterns and dietary energy according to the four feeding treatments. Analyses were conducted within each Period and across Periods in order to assess any differential effects of the treatments.

Treatment (feeding regime) design is summarised in Table 3.5.1. There were four treatments for three six-week time Periods over a total of 18 weeks. These treatments included consistent supply of medium protein and medium lipid over the entire season (T1), supply of locally caught sardines only (T4) and either low protein and high lipid progressing to high protein and low lipid (T2) or vice versa (T3). The formulated combinations of the baitfish used in this Trial are described in Section 2.6.

Table 3.5.1 Summary of the treatment design used in Trial 5.

Treatment (Pontoon)	Period 1 0-6 wk	Period 2 7-12 wk	Period 3 13-18 wk
T1 (10)	MP/ML	MP/ML	MP/ML
T2 (11)	LP/HL	MP/ML	HP/LL
T3 (12)	HP/LL	MP/ML	LP/HL
T4 (13)	Local	Local	Local

Note - MP – medium protein; ML – medium lipid; HP – high protein; HL – high lipid; LP – low protein; LL – low lipid; Local – locally caught sardines

General husbandry

Water temperature (T_w) dropped linearly during the Trial and was consistent with T_w profiles from previous years in the tuna offshore farming zone. At the beginning of the Trial, T_w was 18.1^{o}C, falling to 16.8^{o}C at the beginning of Period 2, 14.6^{o}C at the beginning of Period 3 and dropping further to 13.8^{o}C at the completion of Period 3 (Figure 3.5.1).

Close attention to baitfish combinations fed during the season resulted in provision of diets that closely matched the planned experimental design. SBT were fed 88% of the intended treatment (feeding regime) in Period 1, 75% in Period 2 and 78.5% in Period 3, due to weather and commercial constraints. Mortality was consistent across all treatment groups (approximately 10% per treatment) and was likely to be a reflection of handling and tagging.

Based on SBT length, the length data suggests that the 879 tagged SBT consisted of mainly two or three year old SBT (Polacheck et al., 2004). Although different ratios of the cohorts were placed into research pontoons due to random allocation, it was assumed when calculating growth rates for the experiment that percentage length and weight increases were similar for year classes (cohorts).

Experimental pontoons were stocked with different percentages of SBT cohorts (Figure 3.5.2). It is recognised that this can lead to analytical errors.

Treatment effects with each Trial Period

Separate analyses were conducted to evaluate the effects of the treatment (feeding regimes) within each Trial Period. The results are summarised in Tables 3.5.2, 3.5.3, and 3.5.4. For each index of SBT condition, the p-value for the ANOVA is reported. The R^2 values are also reported to give an indication of effect size (noting that the number of data points varied between treatments and Periods). If the overall effect was statistically significant, post hoc comparison of the means was conducted to evaluate where the differences occurred. For each significant pair in the table, the treatment number of the smaller category appears under the category with the larger mean. Caution was used in interpreting the results given the large number of comparisons and the concomitant increase in Type I error rate. Rather, the overall trends were examined rather than placing undue emphasis on the specific significance test results.

The condition index (CI) did not vary between treatments at Period 1. However, by Period 2, T4 had a lower CI than the rest of the other feeding regimes, and at Period 3, T1 had a higher CI than both T3 and T4 (Figure 3.5.3). The increase in length (Figure 3.5.4) and in weight (Figure 3.5.5) did not vary between treatments for any of the Periods.

Variance in the specific growth rate (SGR) was not seen until Period 3, at which time T1 had a higher mean SGR than either T3 or T4, and T2 had a higher SGR than T4 (Figure 3.5.6). It also appears from the figure that the SGR for T4 in Period 1 was considerably lower than the other treatments.

The differences in cage consumption per day (expressed as intake % of body weight) according to treatment were variable depending on the Period (Figure 3.5.7). No differences were seen in Period 1. For Period 2, the cage consumption for T1 and T2 exceeded that of T4. In contrast, in Period 3, the cage consumption of T1 and T4 exceeded that of T3. In Period 1, the intake per SBT (kg) was higher in T3 and T4 than in T1 and T2. In Period 3, the consumption of T4 exceeded that of the other three treatments (Figure 3.5.8).

Table 3.5.2 Summary of the differences in SBT condition by treatment in **Period 1**. For each significant pair, the treatment number of the smaller category appears as a superscript under the category with the significantly larger mean.

Index	F	df	Sig.	R^2	Treatment 1 (MP/ML)	Treatment 2 (LP/HL)	Treatment 3 (HP/LL)	Treatment 4 (Local)
Condition Index	1.367	3,36	0.268	0.102	22.2 ±0.88	21.9 ±0.6	20.6 ±0.79	20.3 ±0.87
Weight increase (kg)	0.635	3,35	0.598	0.052	4.67 ±1.0	4.56 ±0.63	4.31 ±1.11	2.92 ±1.18
Length increase (cm)	0.530	3,35	0.664	0.043	2.05 ±0.54	2.35 ±0.63	2.15 ±0.68	1.33 ±0.47
Specific Growth Rate	1.686	3,35	0.188	0.126	0.47 ±0.1	0.47 ±0.07	0.37 ±0.09	0.22 ±0.08
Daily cage intake (FI)	1.958	3,155	0.123	0.037	9.67 ±1.78	8.73 ±1.51	9.38 ±2.08	9.51 ±2.08
Daily SBT intake (kg)	7.662***	3,156	< 0.001	0.128	1.67 ±0.25	1.6 ±0.21	$1.82^{1,2}$ ±0.33	$1.92^{1,2}$ ±0.46
Protein (g/100 g)	5.830**	3,36	0.002	0.327	22.65^{3} ±0.63	23.17^{3} ±0.42	20.05 ± 0.92	20.31^{3} ±0.45
Lipid (g/100 g)	3.282*	3,36	0.032	0.215	11.15 ±2.03	15.49^{4} ±1.39	12.19 ±2.48	7.08 ±1.57
Moisture (g/100 g)	3.193*	3,36	0.035	0.210	64.45 ±2.3	59.98 ±1.08	66.13^{2} ±2.02	67.54^{2} ±1.73

*Note. Separate ANOVAs were conducted for each SBT condition index. *p<.05, **p< .01, ***p<.001. Mean difference results are based on two-sided tests with significance level of 0.05 (no correction).*

Table 3.5.3 Summary of the differences in SBT condition by treatment in **Period 2**. For each significant pair, the treatment number of the smaller category appears as a superscript under the category with the significantly larger mean.

Index	F	df	Sig.	R^2	Treatment 1 (MP/ML)	Treatment 2 (MP/ML)	Treatment 3 (MP/ML)	Treatment 4 (Local)
Condition Index	6.851***	3,116	<0.001	0.151	25.05^4 ±0.41	25.25^4 ±0.29	24.76^4 ±0.27	23.39 ±0.29
Weight increase (kg)	1.733	3,104	0.165	0.048	7.62 ±0.54	8.52 ±0.4	8.07 ±0.39	7.23 ±0.4
Length increase (cm)	0.180	3,104	0.910	0.005	3.39 ±0.55	3.57 ±0.38	3.25 ±0.26	3.19 ±0.45
Specific Growth Rate	2.215	3,104	0.091	0.060	0.43 ±0.03	0.46 ±0.02	0.43 ±0.02	0.38 ±0.02
Daily cage intake (FI)	2.986*	3,124	0.034	0.067	7.19^4 ±1.15	7.21^4 ±1.16	6.83 ±1.13	6.47 ±1.14
Daily SBT intake (kg)	1.463	3,124	0.228	0.034	1.49 ±0.24	1.61 ±0.27	1.6 ±0.28	1.6 ±0.31
Protein (g/100 g)	0.710	3,36	0.552	0.056	22.56 ±0.23	22.71 ±0.47	22.71 ±0.56	23.32 ±0.24
Lipid (g/100 g)	0.876	3,36	0.462	0.068	16.12 ±1.19	18.1 ±1.29	17.36 ±0.87	15.84 ±1.15
Moisture (g/100 g)	0.701	3,36	0.557	0.055	59.64 ±1.09	57.81 ±1.16	58.52 ±0.48	59.12 ±0.88

*Note. Separate ANOVAs were conducted for each SBT condition index. *p<.05, **p< .01, ***p<.001. Mean difference results are based on two-sided tests with significance level of 0.05 (no correction). Each significant pair is denoted by a superscript. Weight, length and Specific growth rate are cumulative (i.e. the results are combined for Periods 1 and 2 as no clear measure can be made for the same SBT between treatment Periods).*

Table 3.5.4 Summary of the differences in SBT condition by treatment in **Period 3**. For each significant pair, the treatment number of the smaller category appears as a superscript under the category with the significantly larger mean.

Index	F	df	Sig.	R^2	Treatment 1 (MP/ML)	Treatment 2 (HP/LL)	Treatment 3 (LP/HL)	Treatment 4 (Local)
Condition Index	5.161**	3,117	0.002	0.117	$25.51^{3,4}$ ±0.3	25.03^{4} ±0.22	24.3 ±0.28	24.08 ±0.34
Weight increase (kg)	0.246	3,103	0.864	0.007	9.7 ±0.39	9.26 ±0.34	9.13 ±0.53	9.17 ±0.7
Length increase (cm)	1.745	3.103	0.162	0.048	5.48 ±0.32	5.16 ±0.29	4.83 ±0.4	4.32 ±0.4
Specific Growth Rate	3.270*	3,103	0.024	0.087	$0.37^{3,4}$ ±0.02	0.35^{4} ±0.01	0.32 ±0.01	0.3 ±0.02
Daily cage intake (FI)	2.740*	3,128	0.046	0.060	5.84^{3} ±1.08	5.44 ±0.97	5.2 ±0.88	5.68^{3} ±1.0
Daily SBT intake (kg)	6.385***	3,128	< 0.001	0.130	1.36 ±0.23	1.33 ±0.22	1.26 ±0.21	$1.55^{1,2,3}$ ±0.26
Protein (g/100 g)	0.491	3,36	0.691	0.039	22.47 ±0.37	22.78 ±0.69	22.05 ±0.73	21.91 ±0.36
Lipid (g/100 g)	0.252	3,36	0.869	0.021	20.16 ±1.41	19.42 ±1.86	18.92 ±1.28	20.47 ±0.87
Moisture (g/100 g)	0.551	3,36	0.651	0.044	55.91 ±0.99	56.56 ±1.2	57.53 ±1.19	55.91 ±0.67

*Note. Separate ANOVAs were conducted for each SBT condition index. *p<.05, **p< .01, ***p<.001. Mean difference results are based on two-sided tests with significance level of 0.05 (no correction). Each significant pair is denoted by a superscript. Weight, length and Specific growth rate are cumulative (i.e. the results are combined for Periods 1, 2 and 3 as no clear measure can be made for the same SBT between treatment Periods).*

Significant differences in the protein, lipid and moisture content were found in Period 1. The protein content of the SBT in T3 was lower than the remainder of the groups (Figure 3.5.9) and the lipid content for T3 was higher than T4 (Figure 3.5.10). The moisture content of T2 was lower than T3 and T4 (Figure 3.5.11). No differences in the composition were seen in Periods 2 and 3.

Treatment effects from initial baseline samples

Initial proximate values were calculated from five SBT prior to any treatment (Table 3.5.5). These were compared to the total proximate values obtained in the Trial, according to the feeding treatment. There was a significant overall effect of treatment on the protein values ($F = 13.377$, $df = 4, 120$, $p < 0.001$). Post-hoc testing indicated that the mean protein values for each of the four treatments were significantly higher than the initial values ($p < 0.001$ for each comparison).

Similarly overall values for lipid content varied according to treatment ($F = 6.112$, $df = 4, 120$, $p < 0.001$). Each treatment mean was significantly higher than the baseline mean ($p < 0.001$, for each comparison).

Moisture content decreased significantly as a function of treatment ($F = 13.630$, $df = 4, 120$, $p < 0.001$). Each of the four treatments had significantly lower mean moisture content than the initial baseline values ($p < .001$ for each comparison). Protein differed according to Period ($F = 14.493$, $df = 3, 121$, $p < 0.001$). Each Period differed significantly from baseline ($p < 0.001$ for all). Lipid differed according to Period ($F = 29.008$, $df = 3, 121$, $p < 0.001$). Each Period differed significantly from baseline ($p < 0.002$) for all. Moisture differed according to Period ($F = 49.929$, $df = 3, 121$, $p < 0.001$). Each Period differed significantly from baseline ($p < 0.001$) for all.

Table 3.5.5 Initial mean (±SE) nutrient proximate analysis for SBT in Trial 5. n=5.

Index	Value
Protein (g/100 g)	16.94 ± 1.23
Lipid (g/ 100 g)	4.00 ± 1.14
Moisture (g/100 g)	77.32 ± 0.6

Treatment effects over time

Separate analyses were conducted to evaluate the effects over time for each feeding treatment. The results are summarised in Tables 3.5.6, 3.5.7, 3.5.8, and 3.5.9. For each index of SBT condition, the p-value for the ANOVA is reported. The R^2 values are also reported to give an indication of effect size (it should be noted that the number of data points varied between treatments and Periods). If the overall effect was statistically significant, post hoc comparison of the means was conducted to evaluate where the differences occurred. For each significant pair in the table, the treatment number of the smaller category is shown under the category with the larger mean. Some caution should be used in interpreting these results given the number of comparisons and the concomitant increase in Type I error rate. Rather, the overall trends should be examined instead of placing undue emphasis on the specific significance test results.

For T1 (MP/ML at each Period), there were a number of changes in the indexes of SBT condition over time. The condition index, weight, and length increased over time and were highest during Period 3. No difference was seen in the SGR over the treatment Periods. Consumption rates per cage and per SBT were highest during Period 1 and decreased over time. The lipid content increased while the moisture content decreased. No change was seen in the protein content. FCR remained relatively consistent throughout the Trial.

In T2 (LP/HL – MP/HL – HP/LL), changes were seen in numerous indexes over the treatment Periods. Condition index, weight, and length increased. The SGR and consumption rates decreased from Periods 1 and 2 to Period 3.

No change over time was seen in the protein, lipid or moisture content for this diet treatment. FCR remained relatively the same through Periods 1 and 2 and increased in Period 3.

For T3 (HP/LL – MP/ML – LP/HL), effects over time were seen in all indexes. The condition index, length and weight increased, while the consumption rates decreased. SGR was highest during Period 2. Protein and lipid content increased between Periods 1 and 2, while the moisture content decreased in this time frame. FCR remained relatively the same in Periods 1 and 2 and dramatically increased in Period 3.

Treatment 4 (Local – Local – Local) also had significant changes in all the indexes of SBT condition. The condition index, weight and length each increased between Periods 1 and 2. SGR was highest in Period 2. Consumption rates decreased over time. Protein decreased between Periods 2 and Period 3. Lipid content increased over each Period. Moisture decreased between Periods 1 and 2. FCR started high for the first Period, dropped lower than all other FCRs in Period 2 and increased dramatically for Period 3. Food Conversion Ratios are presented in Figure 3.5.12.

Table 3.5.6 Summary of the differences in SBT condition over each Period for **Treatment 1**. For each significant pair, the treatment number of the smaller category appears under the category with the significantly larger mean.

Index	F	df	Sig.	R^2	Period 1 (MP/ML)	Period 2 (MP/ML)	Period 3 (MP/ML)
Condition Index	9.511***	2,68	< 0.001	0.219	22.21 ±0.88	25.04^{1} ±0.41	25.51^{1} ±0.3
Weight increase (kg)	15.194***	2,54	<0.001	0.360	4.67 ±1.01	7.61^{1} ±0.54	$9.7^{1,2}$ ±0.39
Length increase (cm)	11.449***	2,54	<0.001	0.298	2.05 ±0.54	3.39 ±0.55	$5.48^{1,2}$ ±0.32
Specific Growth Rate	1.522	2,54	0.228	0.053	0.47 ±0.1	0.43 ±0.03	0.37 ±0.02
Daily cage intake (FI)	70.411***	2,102	<0.001	0.580	$9.67^{2,3}$ ±1.78	7.19^{3} ±1.15	5.85 ±1.08
Daily SBT intake (kg)	15.647***	2,102	<0.001	0.235	$1.67^{2,3}$ ±0.25	1.48^{3} ±0.24	1.36 ±0.23
Protein (g/100 g)	0.042	2.27	0.959	0.003	22.65 ±0.63	22.56 ±0.23	22.47 ±0.37
Lipid (g/100 g)	8.136**	2,27	0.002	0.376	11.15 ±2.03	16.12^{1} ±1.19	20.16^{1} ±1.41
Moisture (g/100 g)	7.396**	2,27	0.003	0.354	$64.45^{2,3}$ ±2.3	59.64 ±1.09	55.91 ±0.99

*Note. Separate ANOVAs were conducted for each SBT condition index. *p<.05, **p< .01, ***p<.001. Mean difference results are based on two-sided tests with significance level of 0.05 (no correction). Each significant pair is denoted by a superscript. Weight, length and Specific growth rate are cumulative (i.e. the results are combined for Periods 1, 2 and 3 as no clear measure can be made for the same SBT between treatment Periods).*

Table 3.5.7 Summary of the differences in SBT condition over each Period for **Treatment 2**. For each significant pair, the treatment number of the smaller category appears under the category with the larger mean.

Index	F	df	Sig.	R^2	Period 1 (LP/HL)	Period 2 (MP/ML)	Period 3 (HP/LL)
Condition Index	20.945***	2,67	< 0.001	0.385	21.86 ±0.6	25.25^{1} ±0.29	25.02^{1} ±0.22
Weight increase (kg)	20.961***	2,64	< 0.001	0.396	4.57 ±0.63	8.52^{1} ±0.4	9.26^{1} ±0.34
Length increase (cm)	10.231***	2,64	< 0.001	0.242	2.35 ±0.63	3.57 ±0.38	$5.16^{1,2}$ ±0.29
Specific Growth Rate	7.552**	2,64	0.001	0.191	0.47^{3} ±0.07	0.46^{3} ±0.02	0.35 ±0.01
Daily cage intake (FI)	61.917***	2,102	< 0.001	0.548	$8.73^{2,3}$ ±1.51	7.22^{3} ±1.16	5.44 ±0.97
Daily SBT intake (kg)	15.512***	2,102	< 0.001	0.233	1.63^{3} ±0.21	1.61^{3} ±0.27	1.33 ±0.22
Protein (g/100 g)	0.210	2,27	0.812	0.015	23.17 ±0.42	22.71 ±0.47	22.78 ±0.69
Lipid (g/100 g)	1.701	2,27	0.202	0.112	15.49 ±1.39	18.1 ±1.29	19.42 ±1.86
Moisture (g/100 g)	2.287	2,27	.121	.145	59.98 ±1.08	57.81 ±1.16	56.56 ±1.2

*Note. Separate ANOVAs were conducted for each SBT condition index. *p<.05, **p< .01, ***p<.001. Mean difference results are based on two-sided tests with significance level of 0.05 (no correction). Each significant pair is denoted by a superscript. Weight, length and Specific growth rate are cumulative (i.e. the results are combined for Periods 1, 2 and 3 as no clear measure can be made for the same SBT between treatment Periods).*

Table 3.5.8 Summary of the differences in SBT condition over each Period for **Treatment 3**. For each significant pair, the treatment number of the smaller category appears under the category with the significantly larger mean.

Index	F	df	Sig.	R^2	Period 1 (HP/LL)	Period 2 (MP/ML)	Period 3 (LP/HL)
Condition Index	23.628***	2,67	<0.001	0.414	20.61 ±0.79	24.76^{1} ±0.27	24.31^{1} ±0.28
Weight increase (kg)	12.141***	2,67	<0.001	0.266	4.31 ±1.11	8.07^{1} ±0.39	9.13^{1} ±0.53
Length increase (cm)	9.410***	2,67	<0.001	0.219	2.15 ±0.68	3.25 ±0.26	$4.83^{1,2}$ ±0.4
Specific Growth Rate	5.286**	2,67	0.007	0.136	0.37 ±0.09	0.43^{3} ±0.02	0.32 ±0.01
Daily cage intake (FI)	71.240***	2,102	<0.001	0.583	$9.38^{2,3}$ ±2.08	6.83^{3} ±1.13	5.2 ±0.88
Daily SBT intake (kg)	32.130***	2,102	<0.001	0.387	$1.82^{2,3}$ ±0.33	1.6^{3} ±0.28	1.29 ±0.21
Protein (g/100 g)	3.384*	2,27	0.049	0.200	20.05 ±0.92	22.71^{1} ±0.56	22.05 ±0.73
Lipid (g/100 g)	4.366*	2,27	0.023	0.244	12.16 ±2.48	17.36^{1} ±0.87	18.92^{1} ±1.28
Moisture (g/100 g)	11.592***	2,27	<0.001	0.462	$66.13^{2,3}$ ±2.02	58.52 ±0.48	57.53 ±1.19

*Note. Separate ANOVAs were conducted for each SBT condition index. *p<.05, **p< .01, ***p<.001. Mean difference results are based on two-sided tests with significance level of 0.05 (no correction). Each significant pair is denoted by a superscript. Weight, length and Specific growth rate are cumulative (i.e. the results are combined for Periods 1, 2 and 3 as no clear measure can be made for the same SBT between treatment Periods).*

Table 3.5.9 Summary of the differences in SBT condition over each Period for **Treatment 4**. For each significant pair, the treatment number of the smaller category appears under the category with the significantly larger mean.

Index	F	df	Sig.	R2	Period 1(Local)	Period 2 (Local)	Period 3 (Local)
Condition Index	14.929***	2,67	<.001	.308	20.3^{1} ±0.87	23.38^{1} ±0.29	24.08^{1} ±0.34
Weight increase (kg)	14.928***	2,57	<.001	.344	2.92 ±1.18	7.23^{1} ±0.4	$9.17^{1,2}$ ±0.7
Length increase (cm)	7.135**	2,57	.002	.200	1.33 ±0.47	3.19^{1} ±0.45	4.32^{1} ±0.4
Specific Growth Rate	5.002*	2,57	.010	.149	0.22 ±0.08	$0.38^{1,3}$ ±0.02	0.3 ±0.02
Daily cage intake (FI)	63.816***	2,101	<.001	.558	$9.51^{2,3}$ ±2.08	6.47^{3} ±1.14	5.68 ±1.0
Daily SBT intake (kg)	12.421***	2,102	<.001	.196	$1.92^{2,3}$ ±0.46	1.6 ±0.31	1.52 ±0.26
Protein (g/100 g)	5.295*	2,27	.011	.282	23.39^{3} ±0.45	23.32^{3} ±0.24	21.91 ±0.36
Lipid (g/100 g)	30.533***	2,27	<.001	.693	7.08 ±1.57	15.84^{1} ±1.15	$20.47^{1,2}$ ±0.87
Moisture (g/100 g)	25.725***	2,27	<.001	.656	$67.54^{2,3}$ ±1.73	59.12 ±0.88	55.91 ±0.67

*Note. Separate ANOVAs were conducted for each SBT condition index. *p<.05, **p< .01, ***p<.001. Mean difference results are based on two-sided tests with significance level of 0.05 (no correction). Each significant pair is denoted by a superscript. Weight, length and Specific growth rate are cumulative (i.e. the results are combined for Periods 1, 2 and 3 as no clear measure can be made for the same SBT between treatment Periods).*

Analysis of Visceral Warming

This Trial was set up with four feeding regimes over the course of a growing season, divided into three Periods (Table 3.5.9). These regimes included consistent supply of medium protein and medium lipid over the entire season (T1), supply of locally caught sardines only (T4) and either low protein and high lipid progressing to high protein and low lipid (T2) or vice versa (T3). The formulated combinations of the baitfish used in this Trial are described in Section 2.6.

Differences in visceral warming according to feeding treatment and Trial Period

Analyses were conducted to explore whether the feed measure (FM) differed as a result of the treatment group and Period. Analyses were conducted within each Period to compare treatments, and within each treatment to compare effects over time.

Treatment effects within Periods

In Period 1, there were no differences in FM values according to treatment ($p = 0.401$). Significant treatment effects on FM were seen in Period 2 ($F = 6.391$, $df = 3, 99$, $p = 0.001$). Post hoc testing revealed that the FM mean for T3 was lower than T1, T2 and T4 (all $p < 0.05$). Significant treatment effects were also observed in Period 3 ($F = 7.439$, $df = 3, 114$, $p < 0.001$). Post hoc comparisons indicated that T3 had a significantly lower FM mean than T1 ($p = 0.005$), T2 ($p = 0.014$) and T4 ($p < 0.001$). No other comparisons were found to have significant relationships.

In the pooled data (across all Periods), there was a significant overall effect of treatment on the FI ($F = 11.629$, $df = 3, 336$, $p < 0.001$). Post-hoc analyses showed that T3 differed significantly from all other treatments ($p < 0.002$).

As seen in Figure 3.5.14, the mean FM for T3 was lower than the other treatments at each Period and overall (although not significantly lower in Period 1). This was interesting as in Period 2, the first three treatments were given the same feed combinations. Differences were therefore not expected.

Treatment effects over time.

For T1, significant effects were seen over time ($F = 14.487$, $df = 2, 48$, $p < 0.001$). The FM for Period 1 was significantly lower than the mean values in either Period 2 or Period 3 ($p < 0.001$). In T1, the SBT were fed a consistent diet across the Periods. Differences according to Period were not seen for T2 ($p = 0.373$) or T3 ($p = 0.826$). In T4, there was a significant effect of Period ($F = 3.893$, $df = 2, 86$, $p = 0.024$). The mean FM for Period 3 was higher than Period 1 ($p = 0.007$). T4 comprised a consistent diet of local sardines across Periods.

Taken together the results indicate higher FM values at time Period 3 compared to time Period 1 for T1 (consistent MP/ML) and T4 (consistent local sardines). No discernible changes over time were seen for T2 or T3 (progressive diets). In the pooled data (across treatments), the mean FM values did not differ according to Period ($p = 0.123$).

Relationship between intake and visceral warming

Analyses were conducted to determine the relationship between intake and FM. Feed intake was calculated as a percentage of body weight per day (FI). For these analyses, the data were pooled across treatments. Overall, there was no relationship between FI and FM ($F = 1.467$, $df = 1, 338$, $p = 0.227$). However, investigation of the relationship according to T_w revealed interesting trends.

There was no significant relationship between FM and Period ($F = 2.11$, $df = 2,337$, $p = 0.123$). However, there was a significant relationship between intake (FI) and T_w ($F = 1684.321$, $df = 2,337$, $p < 0.001$). As shown in Figure 3.5.15, an interaction between FM and intake FI in relation to T_w appeared evident. The FM increased as T_w decreased, while the FI decreased with colder temperatures. Intake was highest and FM lowest at the warmest T_w (17.1^oC). In contrast, intake was lowest and FM highest at the coldest T_w (14.5^oC) suggesting the presence of an interaction in the relationships between intake FI, FM, and T_w.

The relationships between FM and FI at the three different temperatures used in this Trial are shown in Figure 3.5.16. As this Figure highlights, the relationship between FM and FI appeared to vary according to the T_w.

The slope between BFI and FM was highest at the coldest T_w, and lowest at the warmest T_w.

In other words, the relationship between FM and FI became less direct (i.e. the slopes were flatter and the R^2 values smaller), the higher the T_w. However, as shown by the minimal R^2 values for all the regression lines, it appears that SBT are in a large part regulating their visceral warming independently of FI.

Summary

The results of this Trial were interpreted cautiously given the large number of comparisons and the concomitant increase in Type I error rate. Overall trends were examined rather than specific significance test results. These trends suggested that dietary energy has an influence on CI, SGR, FCR and feed intake but overall did not influence absolute length or weight increase. Changes to flesh proximate composition (protein, lipid and moisture) occurred between Periods for all treatments and there were some changes in flesh composition in the first Period relating to the high lipid diet.

Visceral warming patterns

There were no differences in FM values according to treatment in Period 1. Significant treatment effects were seen in Period 2 and 3 for T3 compared with other treatments. There were different FM changes over time for treatment groups that did not reflect the intake of dietary energy.

In the pooled data (across treatments), the mean FM values did not differ according to Period. However, an interaction between FM and FI in relation to T_w appeared evident. The FM increased as T_w got colder, while the FI decreased with colder temperatures. Feed intake was highest and FM lowest at the warmest T_w (17.1^oC). In contrast, feed intake was lowest and FM highest at the coldest T_w (14.5^oC) suggesting an interaction in the relationships between intake FI, FM, and T_w.

GLMs developed in Trial 2 were applied to the data but were inconclusive. At this time, intake cannot be predicted through available models due to the complexity of factors that appear to influence visceral warming rates.

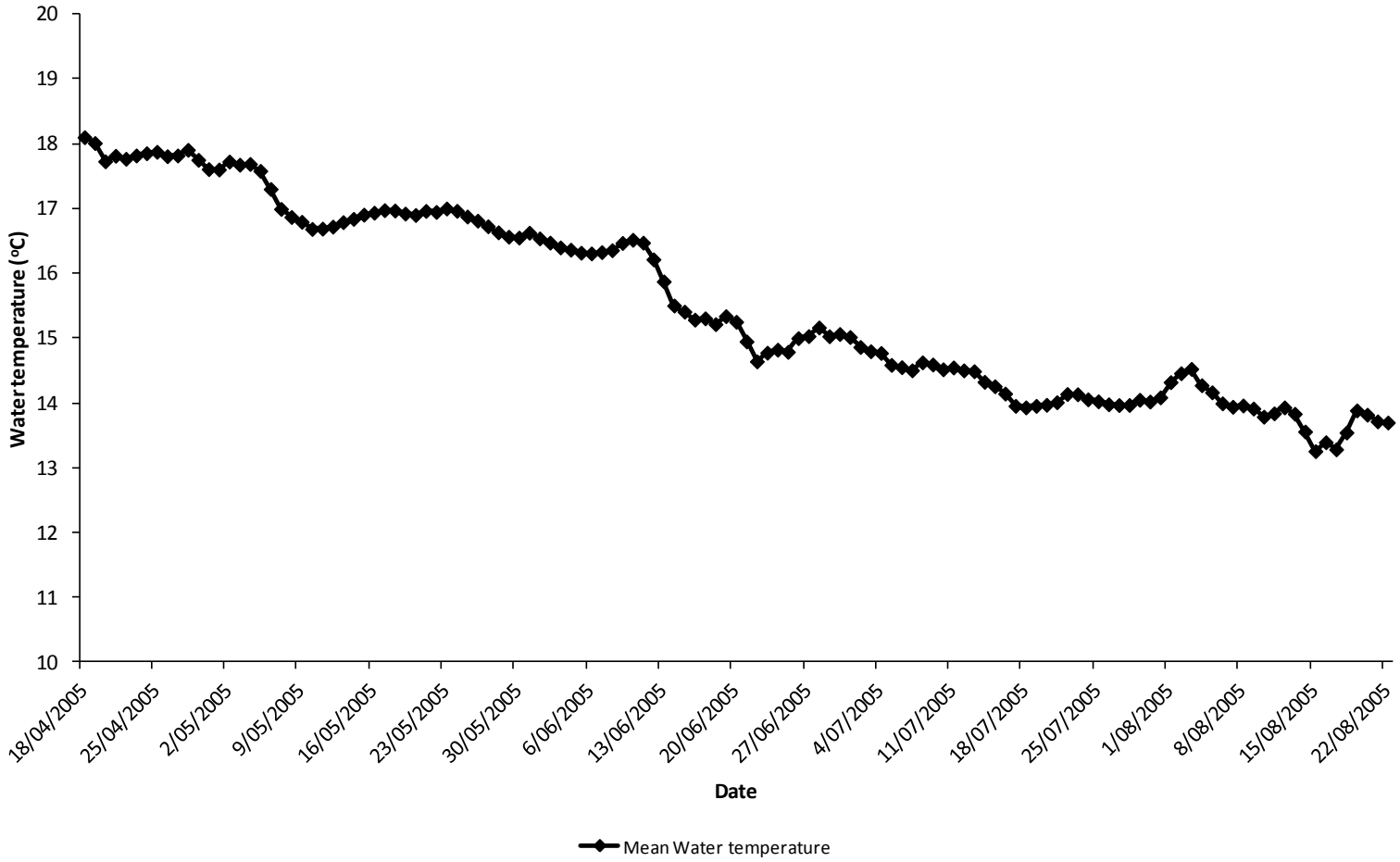


Figure 3.5.1 Mean water temperature (^{o}C) during the experimental Period (date) measured at a depth of 5m using a Vemco data recorder.

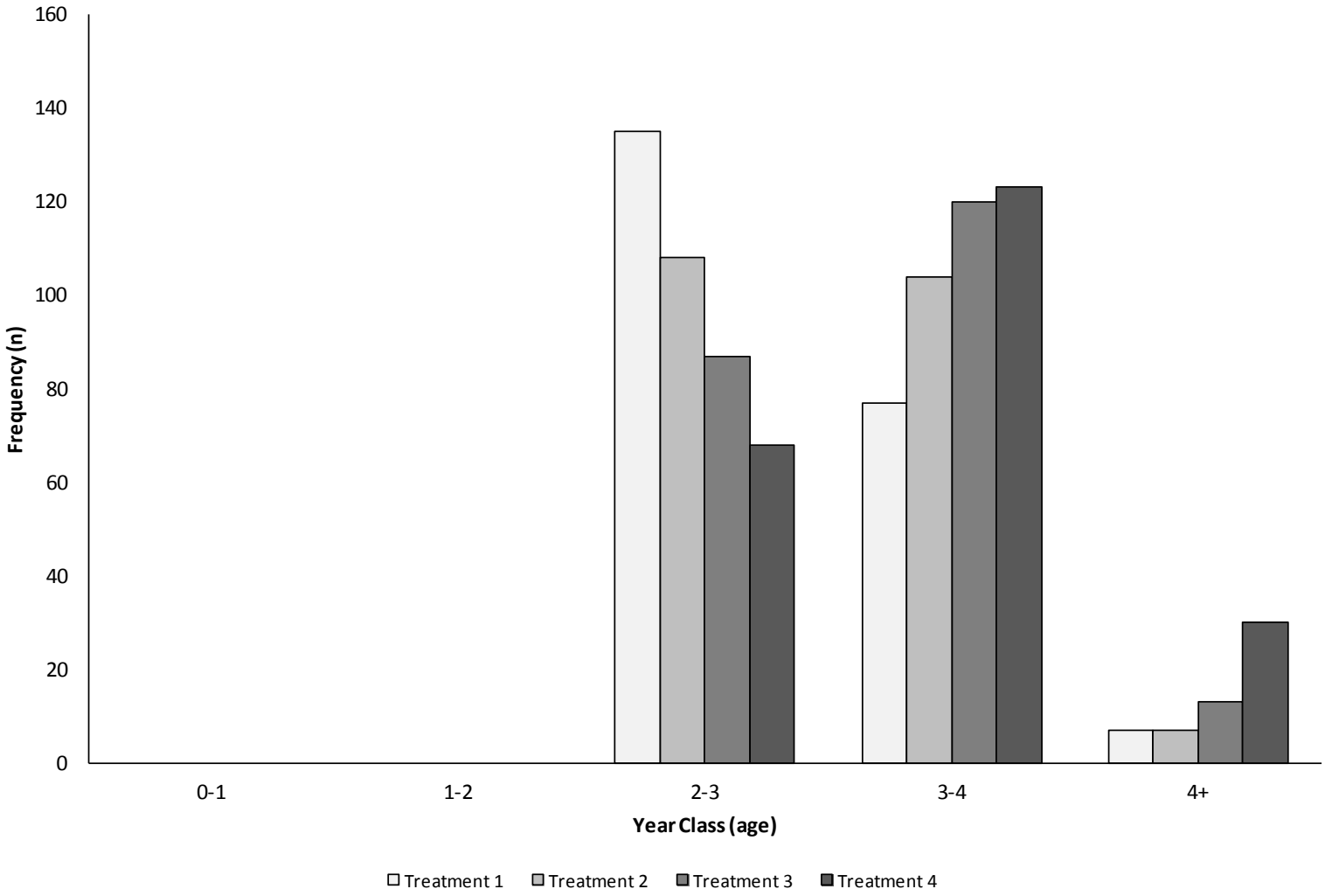


Figure 3.5.2 Histogram showing frequency (n) in response to year class (age) determine by SBT length (cm). Length at age class assessment from CSIRO 2004 SBT stock assessment (Polacheck et al., 2004).

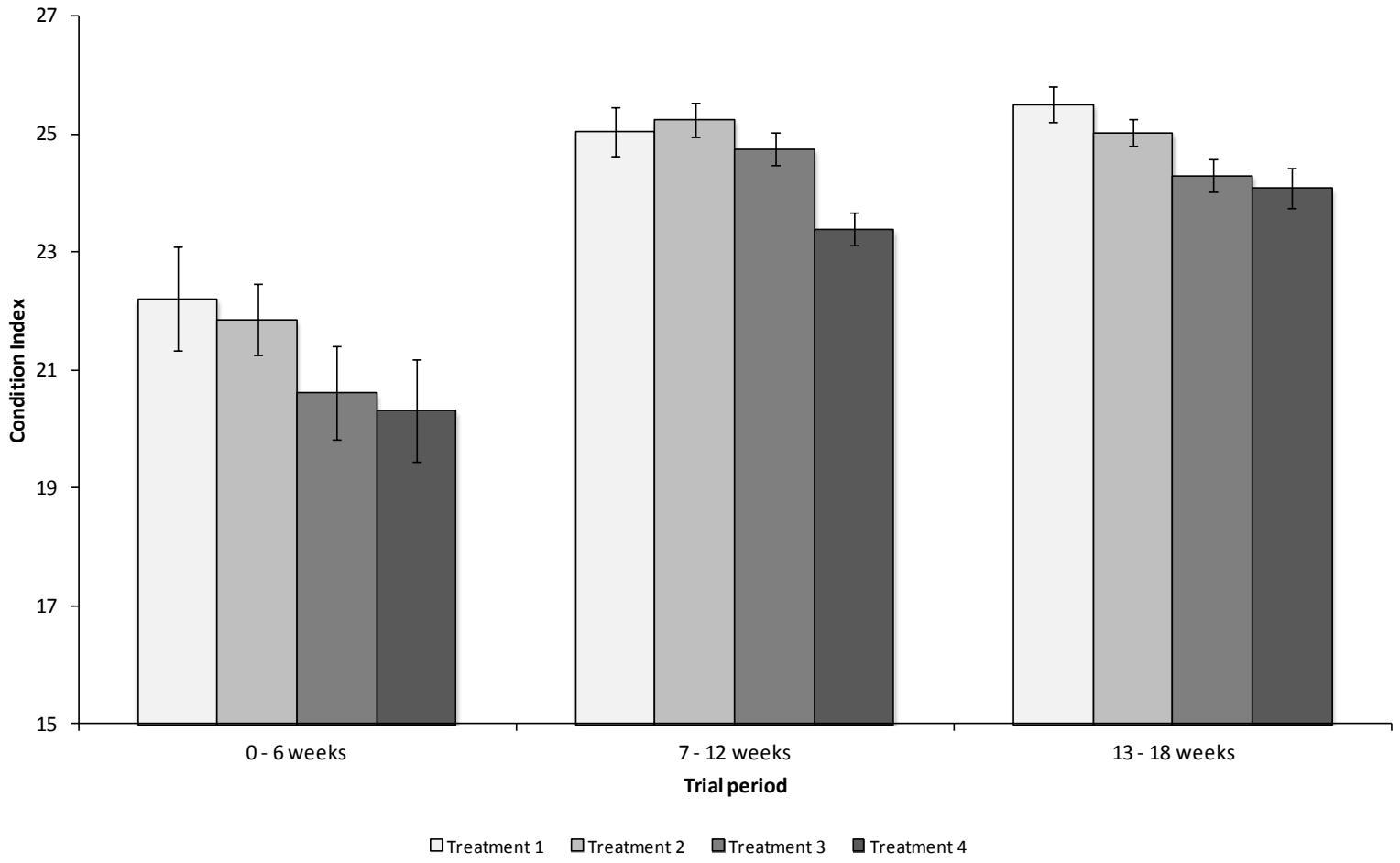


Figure 3.5.3 Mean condition index (weight kg/length m^3) for each treatment during Trial experimental Periods.

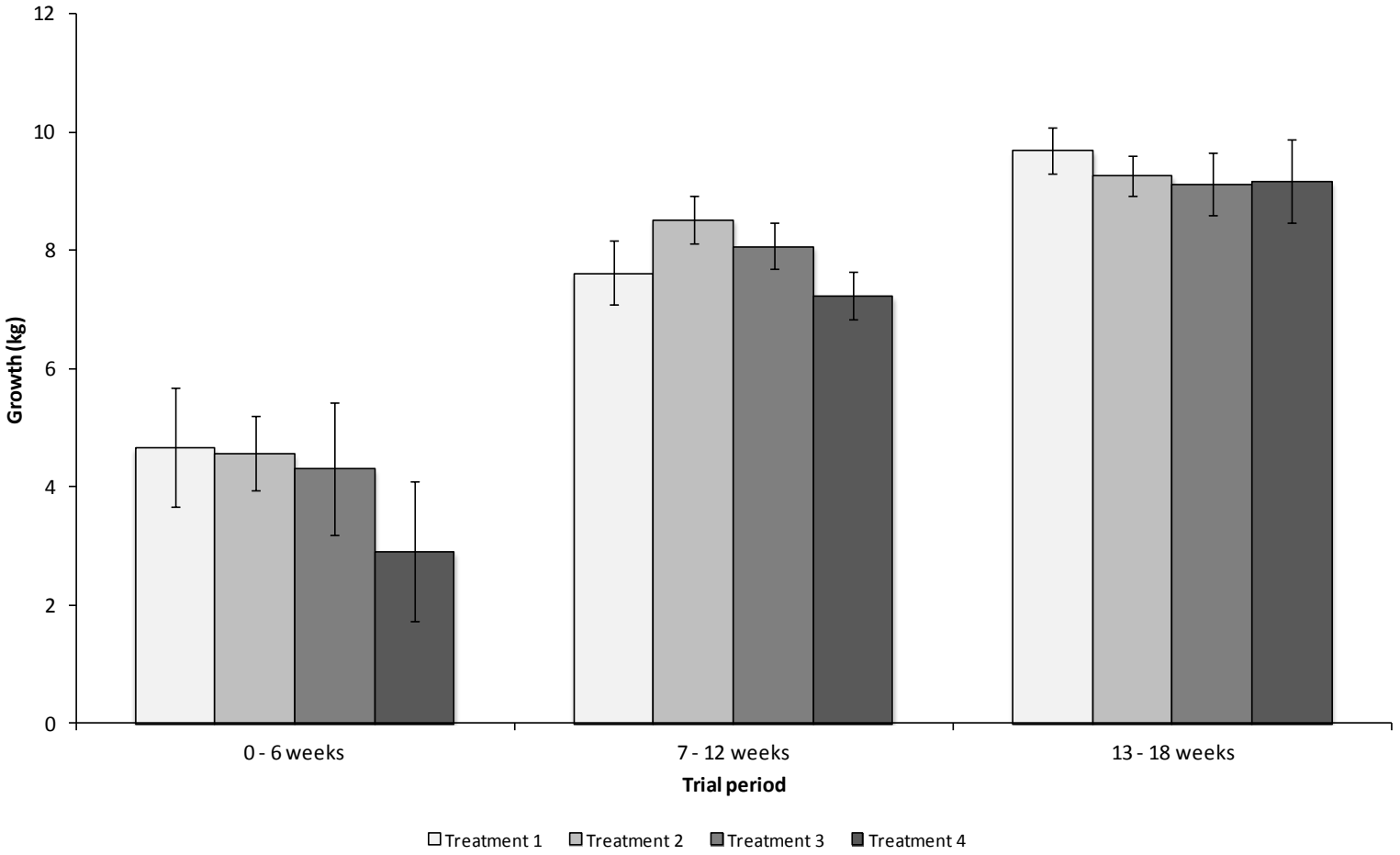


Figure 3.5.4 Cumulative mean weight increase (kg) for each treatment during Trial experimental Periods.

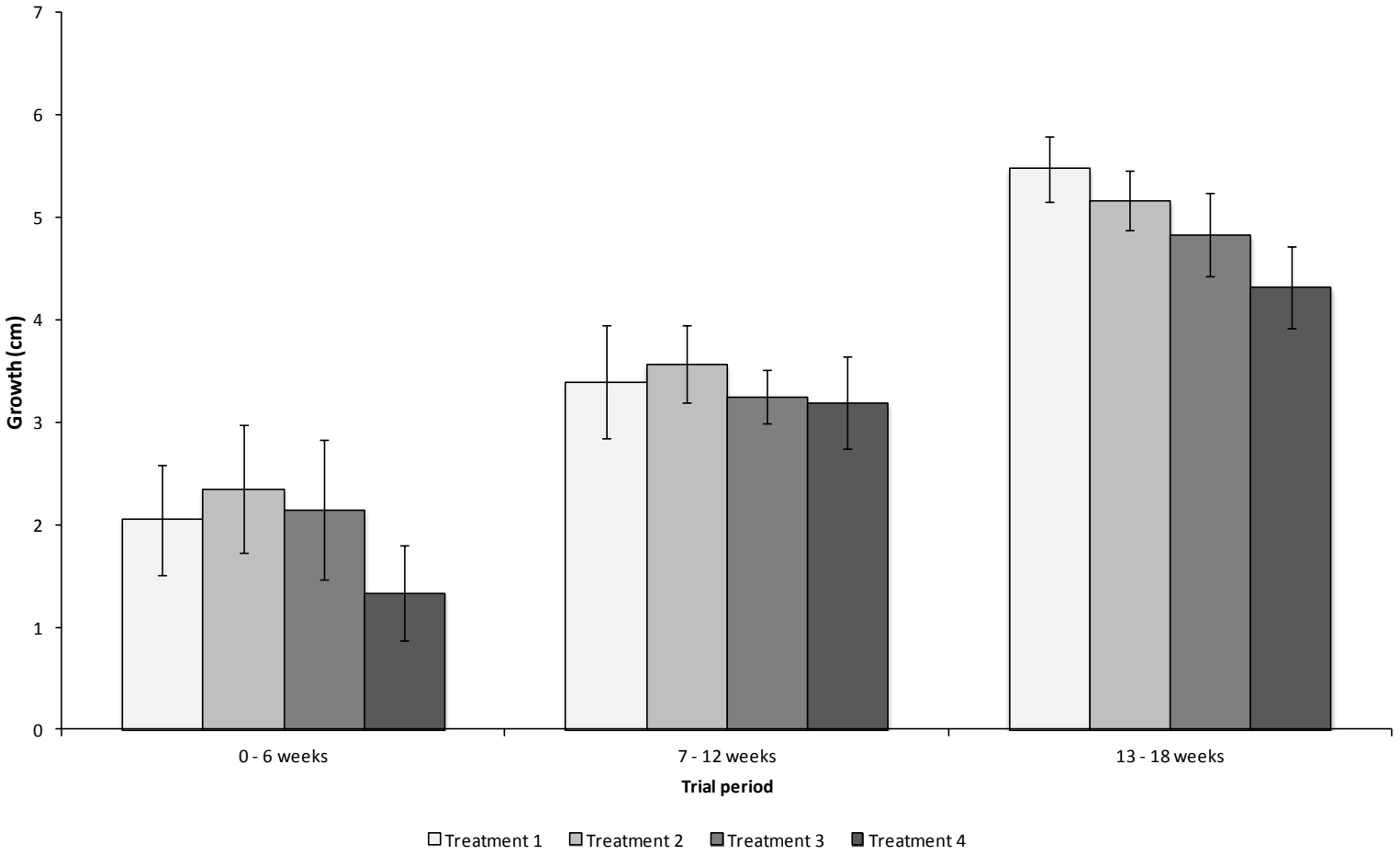


Figure 3.5.5 Cumulative mean length (cm) increase for each treatment during Trial experimental Periods.

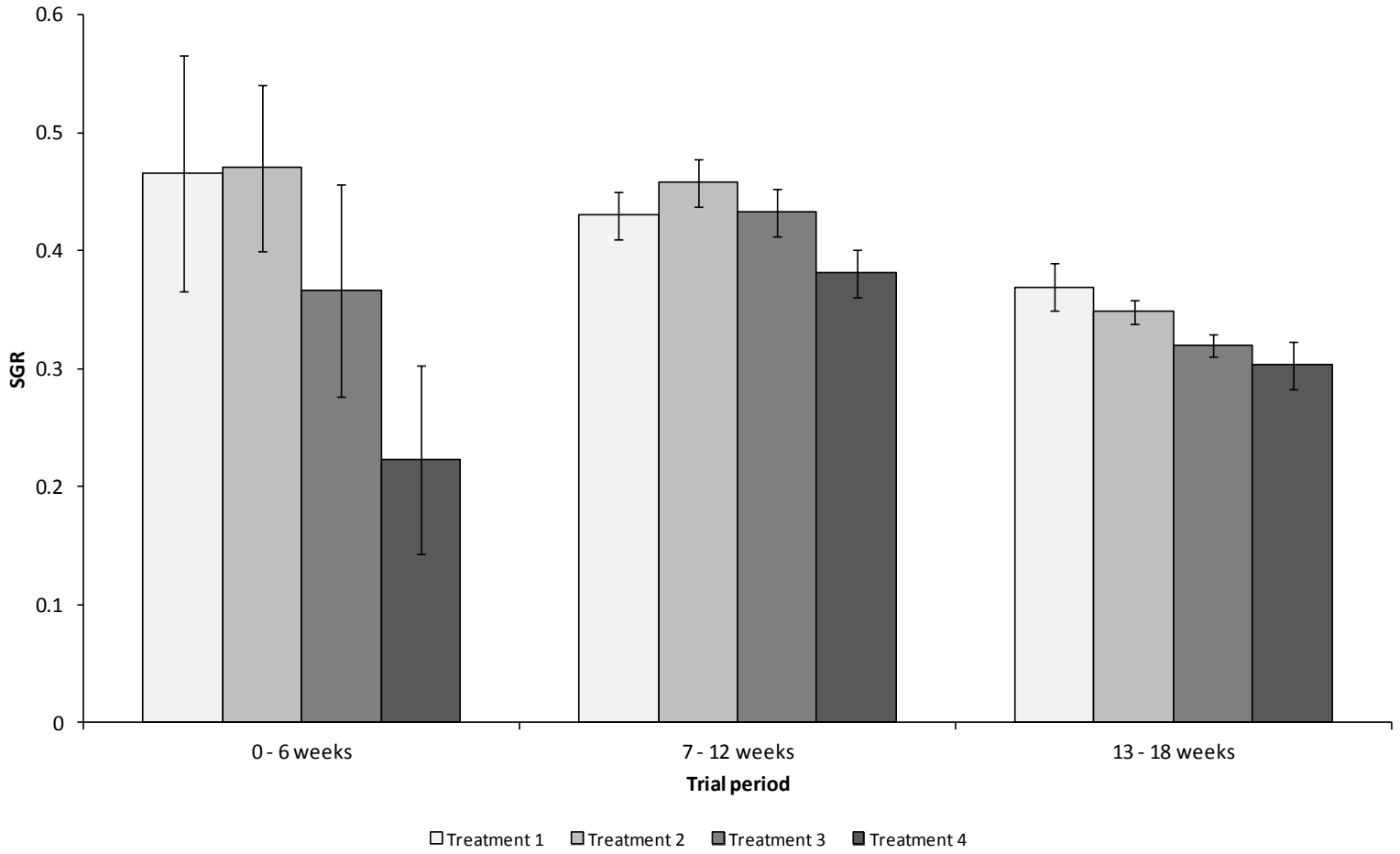


Figure 3.5.6 Cumulative mean specific growth rate (SGR) for each treatment during Trial experimental Periods.

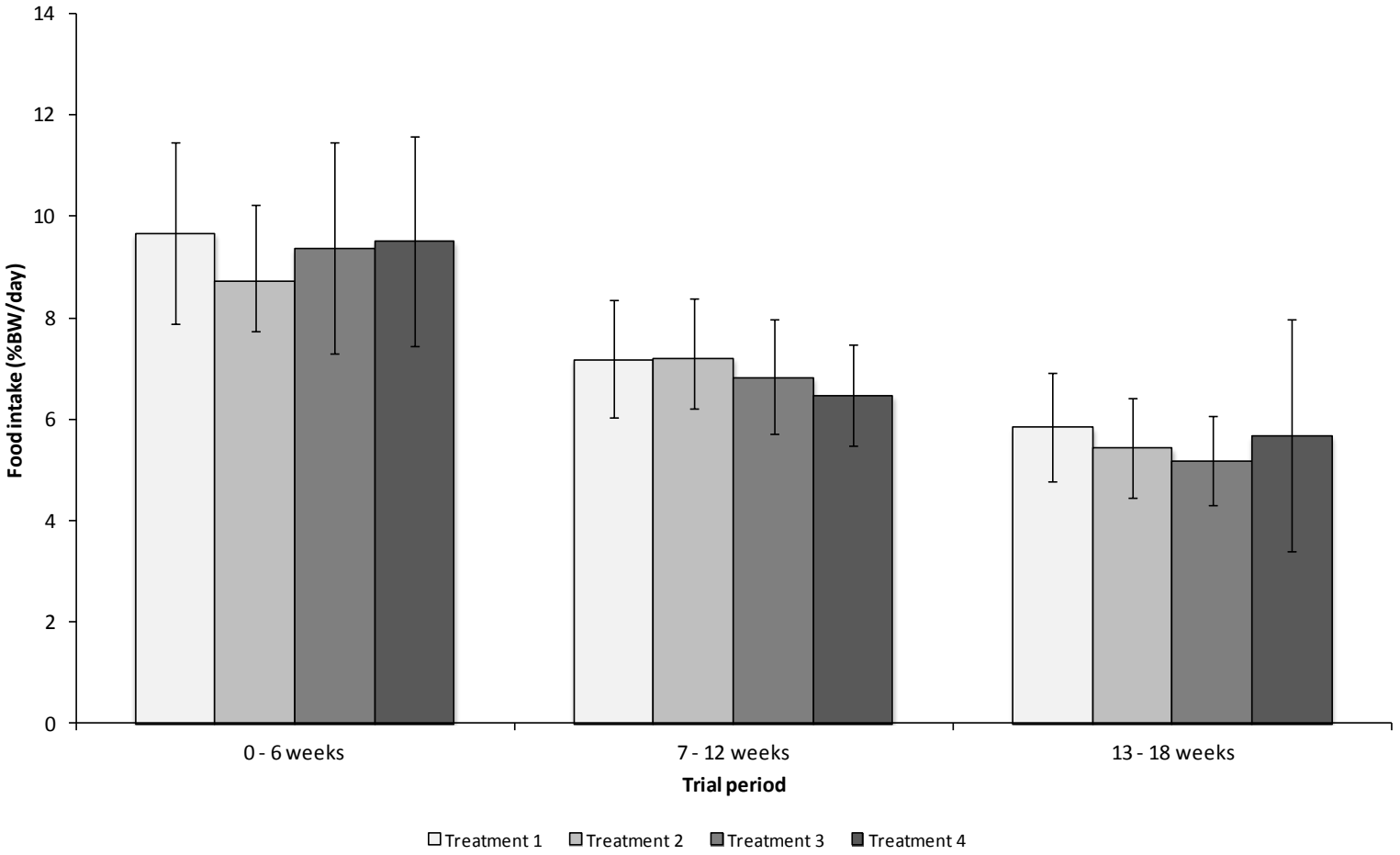


Figure 3.5.7 Mean cage consumption per day (expressed as a % of body weight) for each treatment during each Period of the Trial.

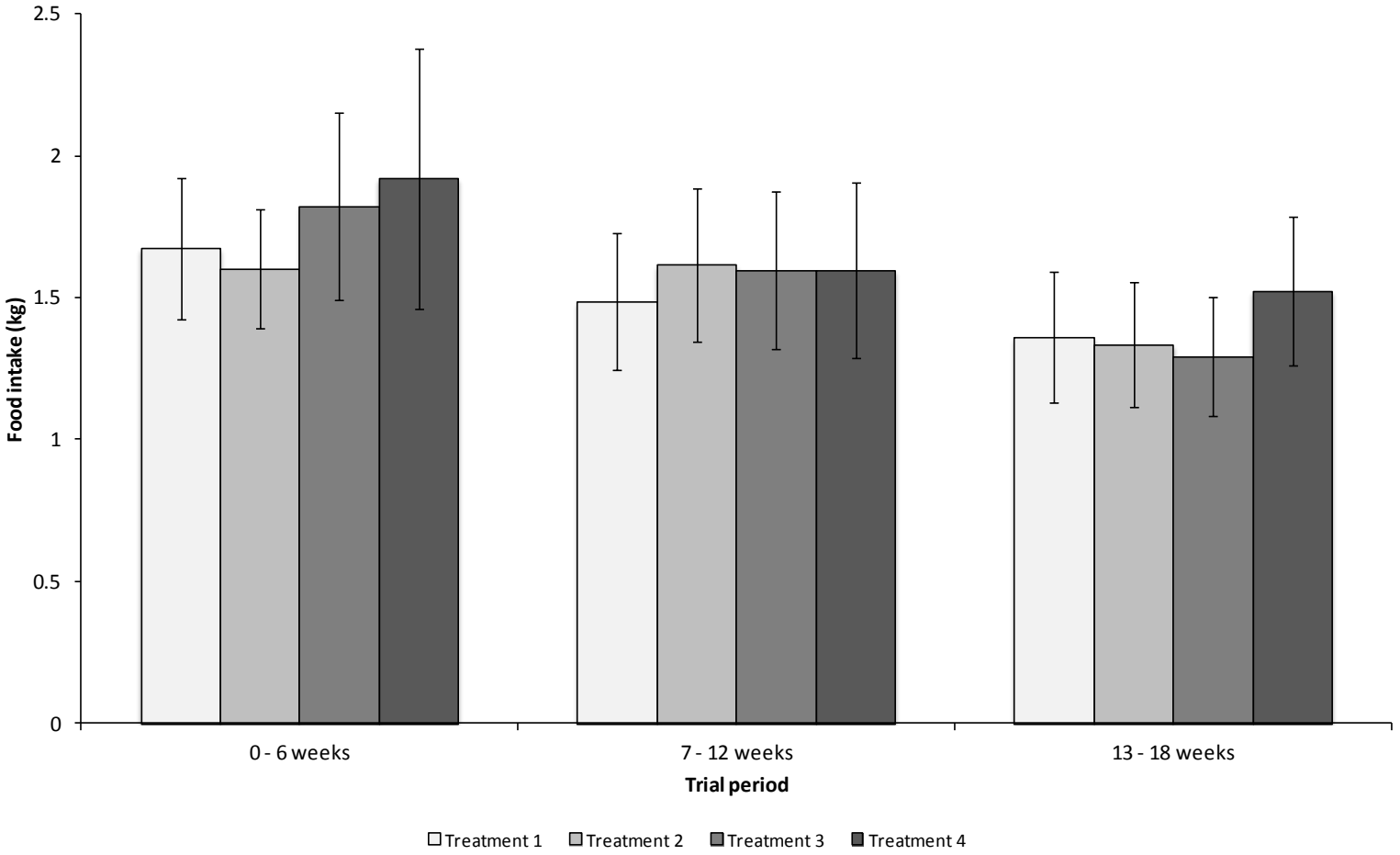


Figure 3.5.8 Mean consumption per SBT per day (kg) for each treatment during Trial experimental Periods.

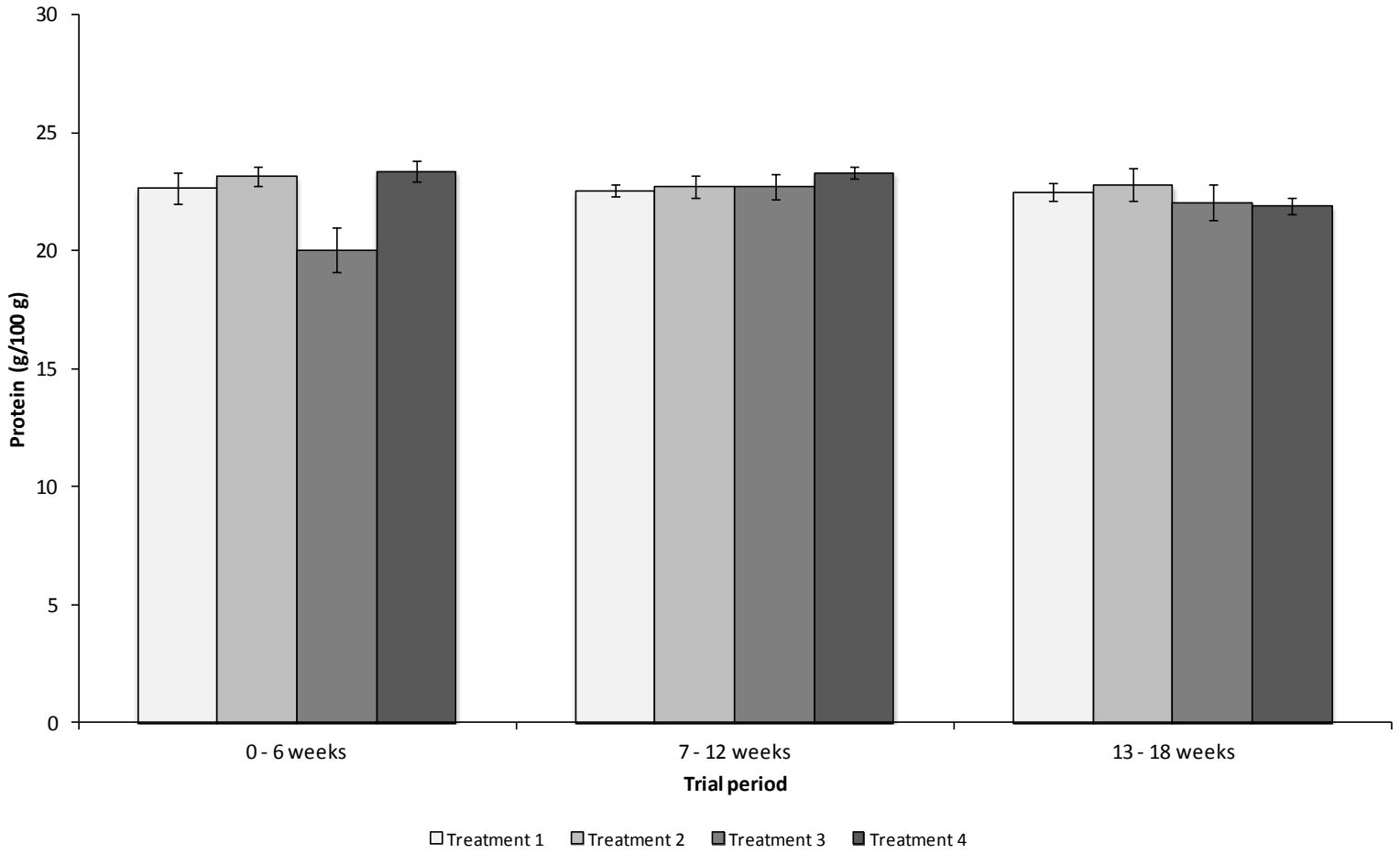


Figure 3.5.9 Mean SBT protein (g/100 g) composition for each feeding each treatment during Trial experimental Periods.

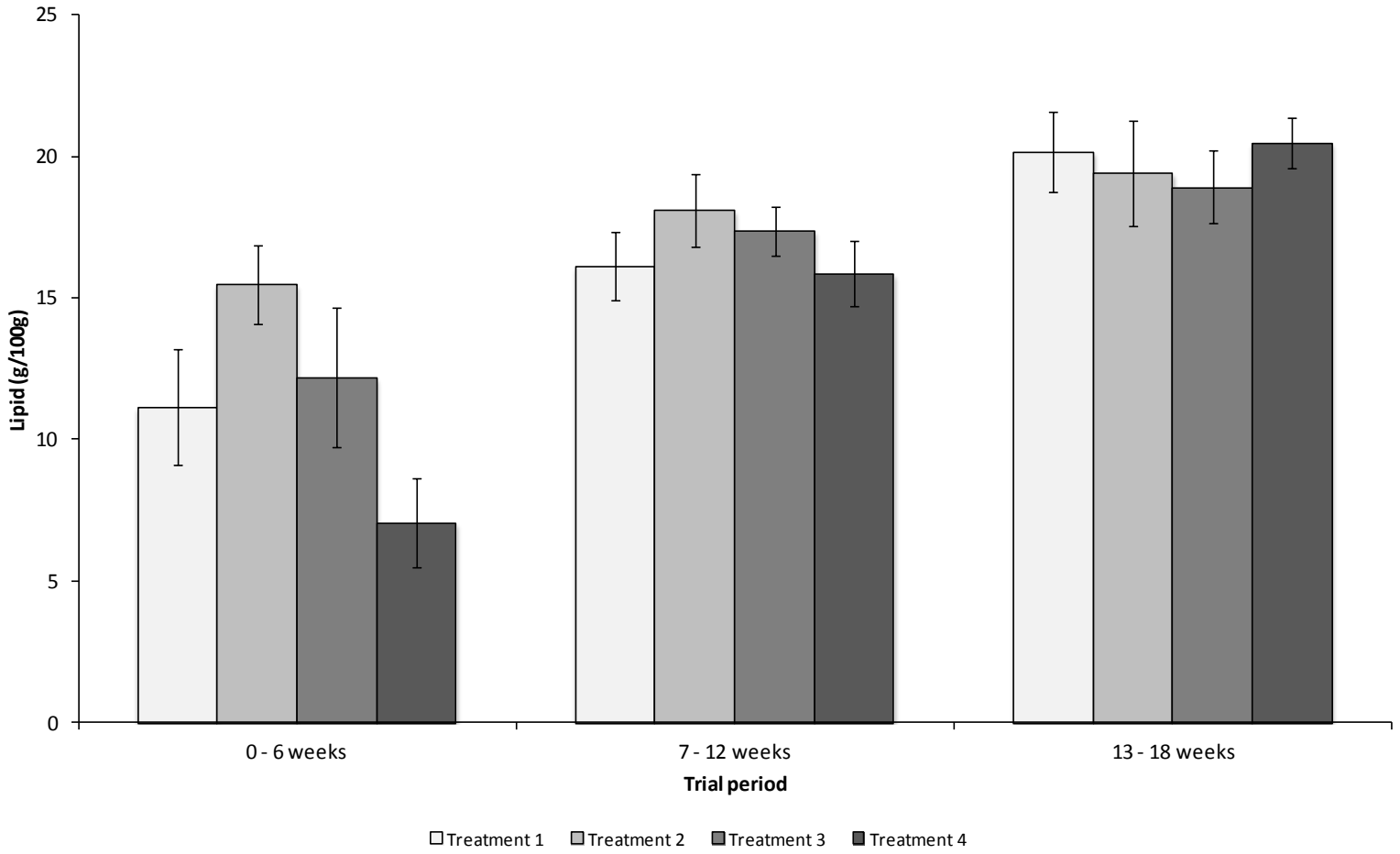


Figure 3.5.10 Mean SBT lipid (g/100 g) composition for each treatment during Trial experimental Periods.

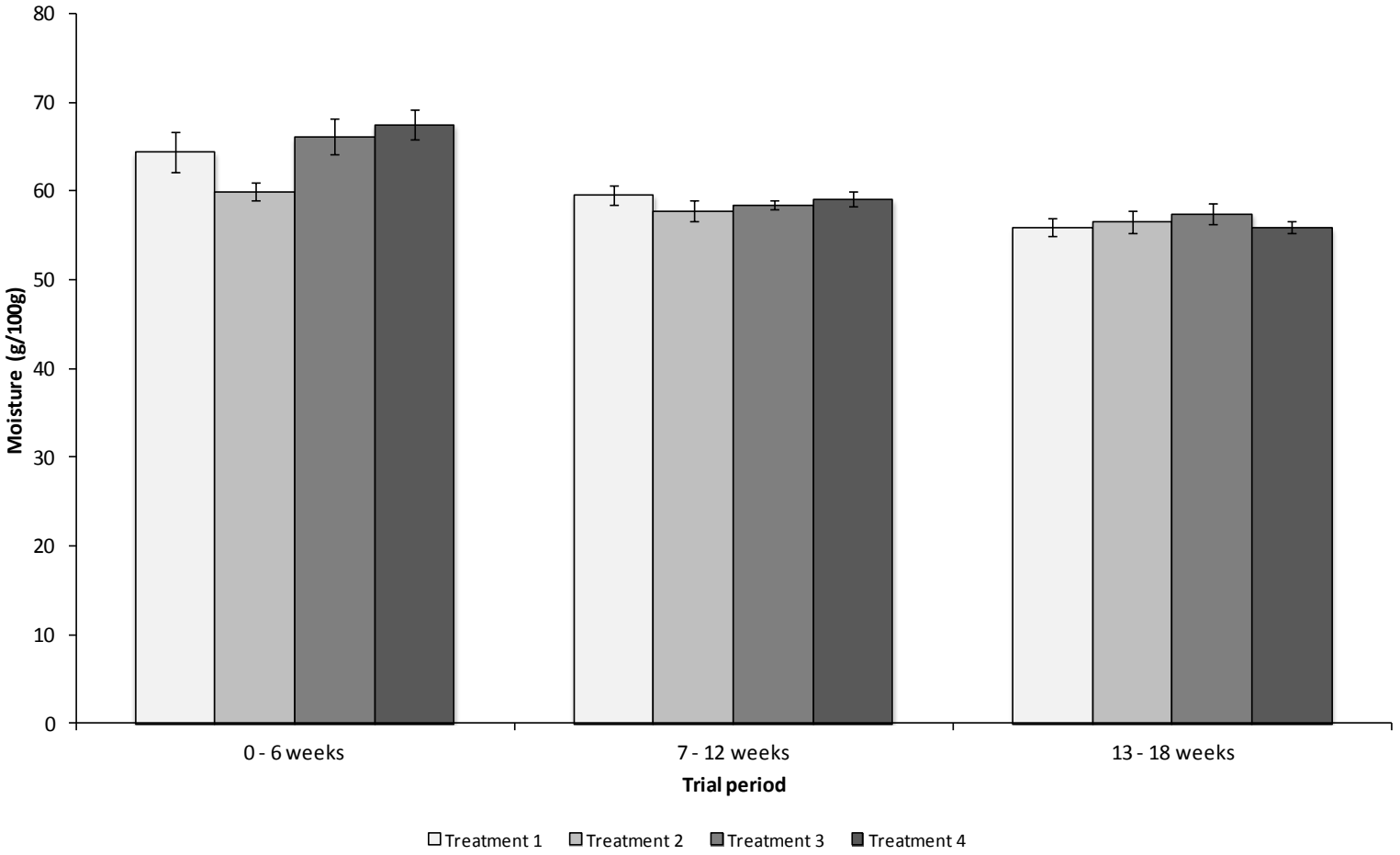


Figure 3.5.11 Mean SBT moisture (g/100 g) composition for each treatment during Trial experimental Periods.

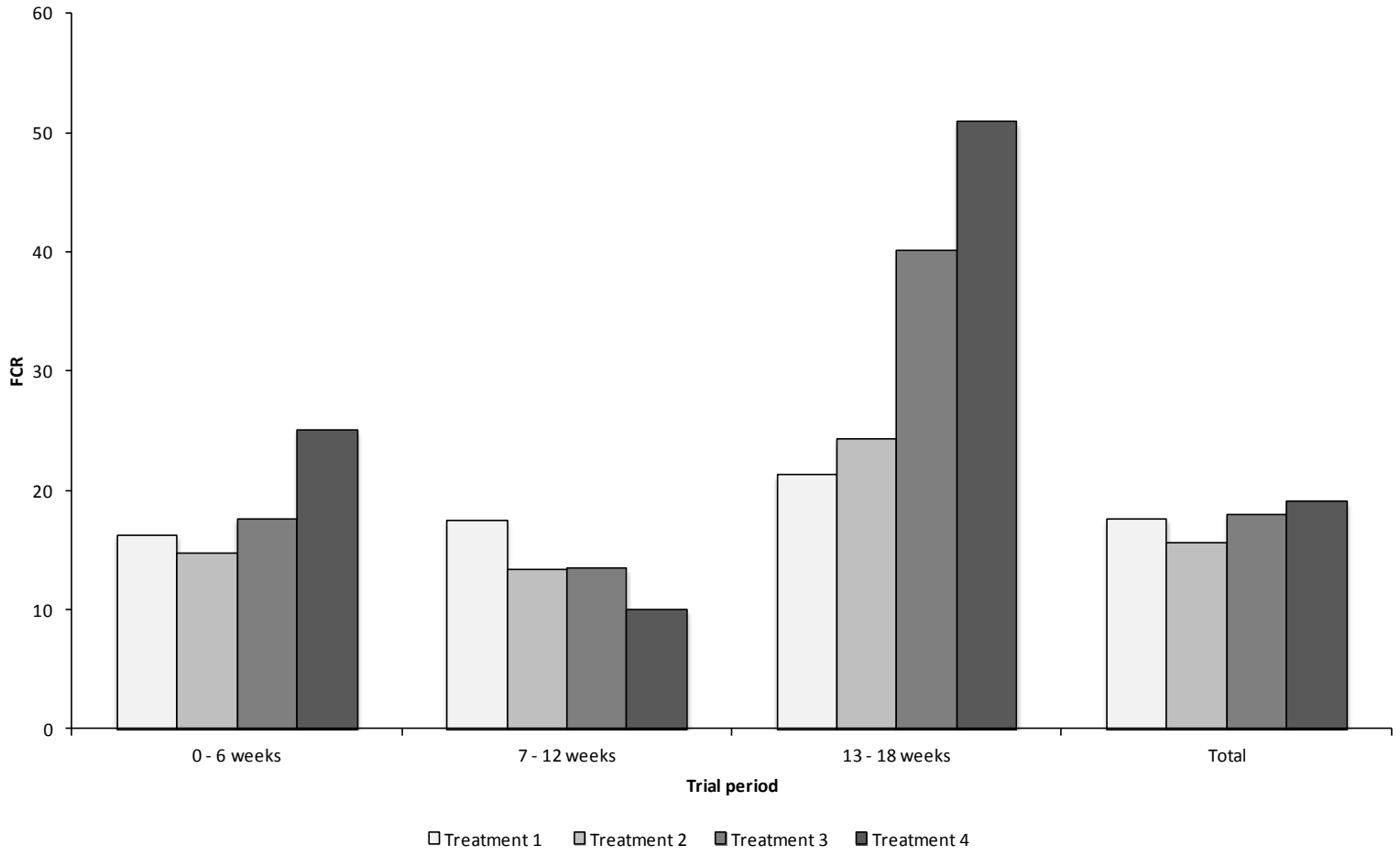


Figure 3.5.12 Food Conversion Ratios (FCR) for each treatment during each treatment during Trial experimental Periods and combined figure for all three Periods.

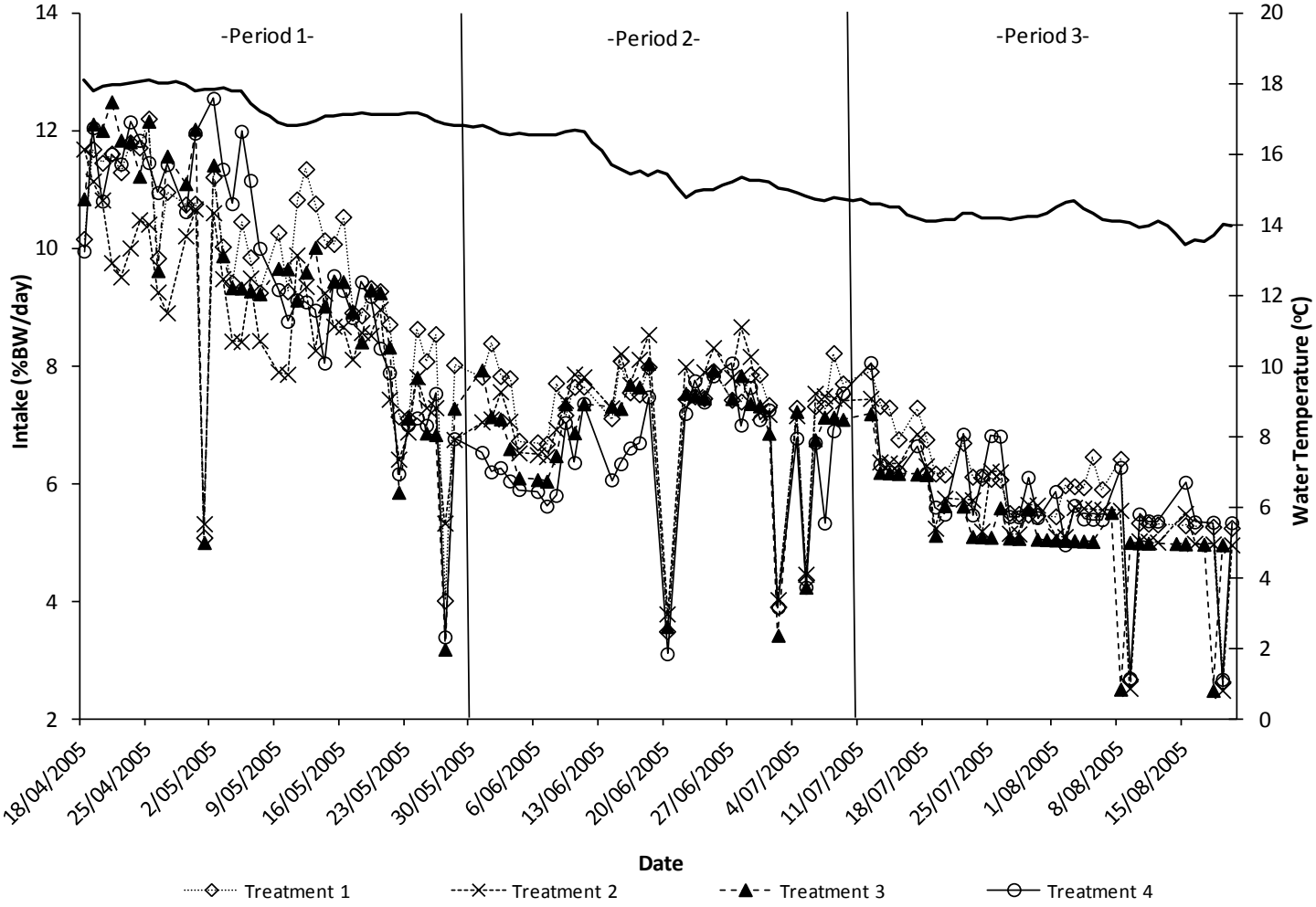


Figure 3.5.13 SBT feed intake (BW/day) plotted against primary Y axis for all treatments in relationship with date for all Trial Periods. Water temperature (^{o}C) is plotted against the secondary Y axis in relationship with date for all Periods. Note – Trial Periods are separated by vertical lines.

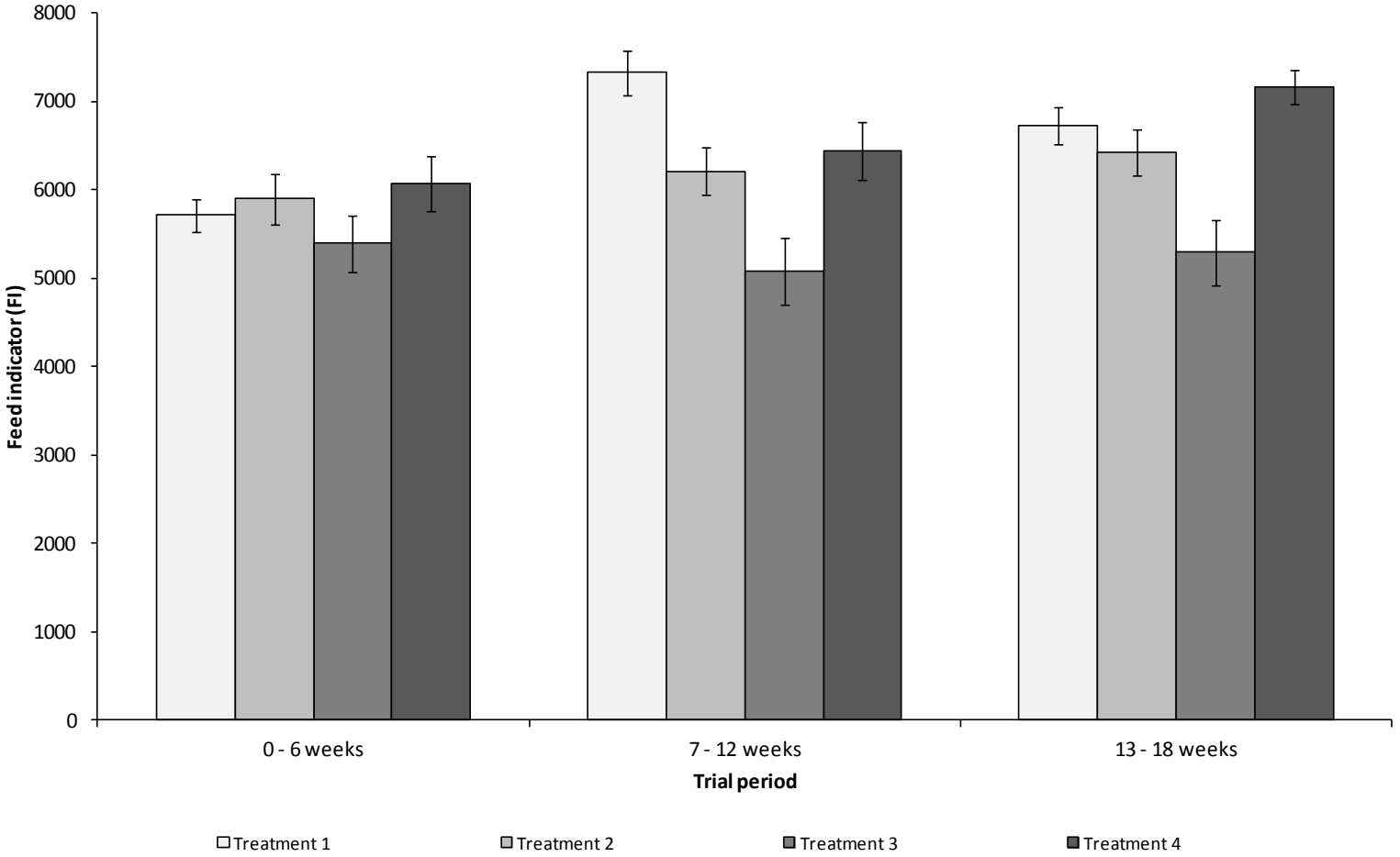


Figure 3.5.14 Mean feed measure for four feeding treatments across three Periods, and overall. Treatment 3 differed significantly from the other treatments for Periods 2, 3, and overall.

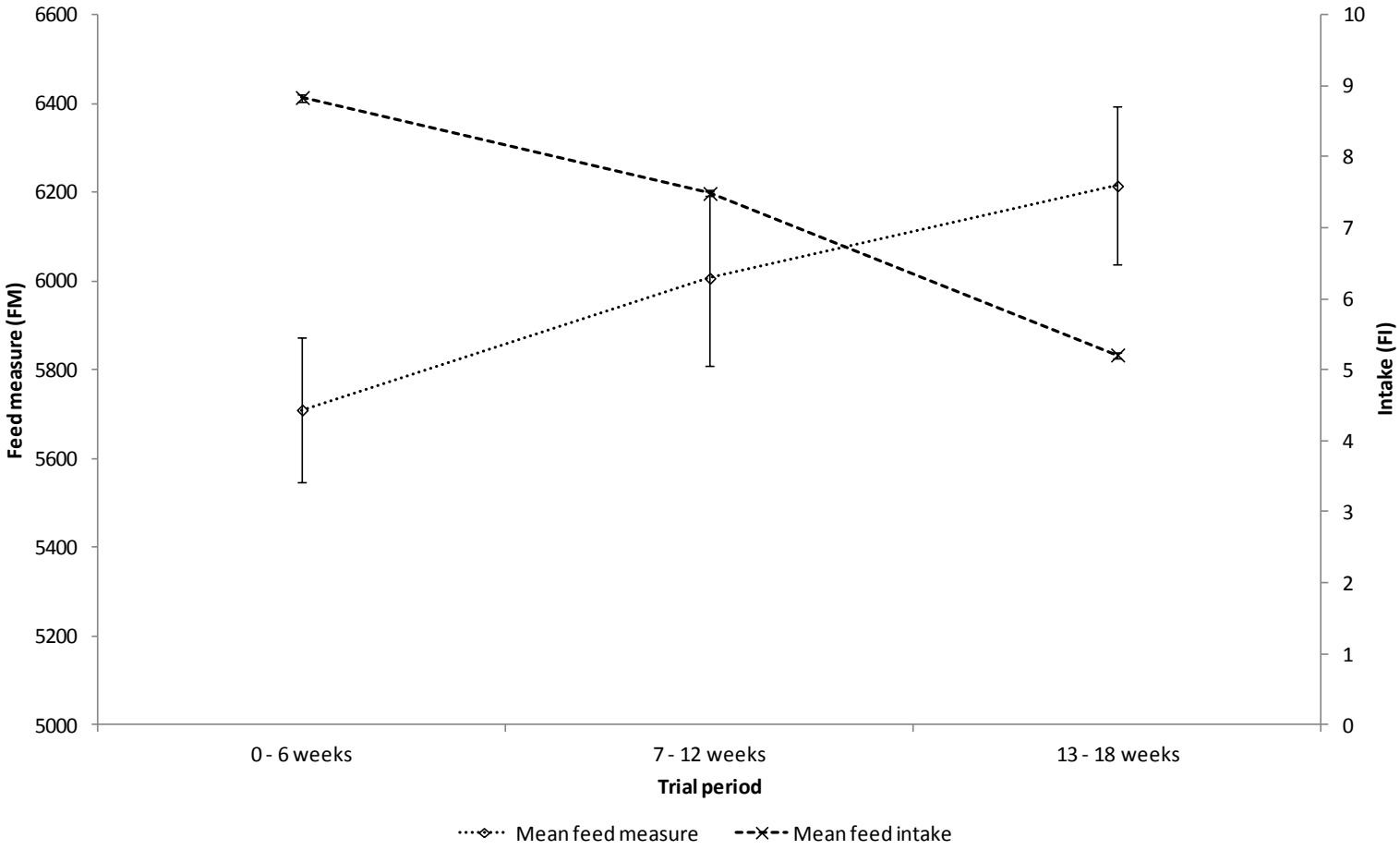


Figure 3.5.15 Mean Feed Measures pooled for all treatments for three Periods, and overall compared with feed intake represented as % body weight per day. There was a clear trend that as water temperature decreases, feed intake decreases and visceral warming increases.

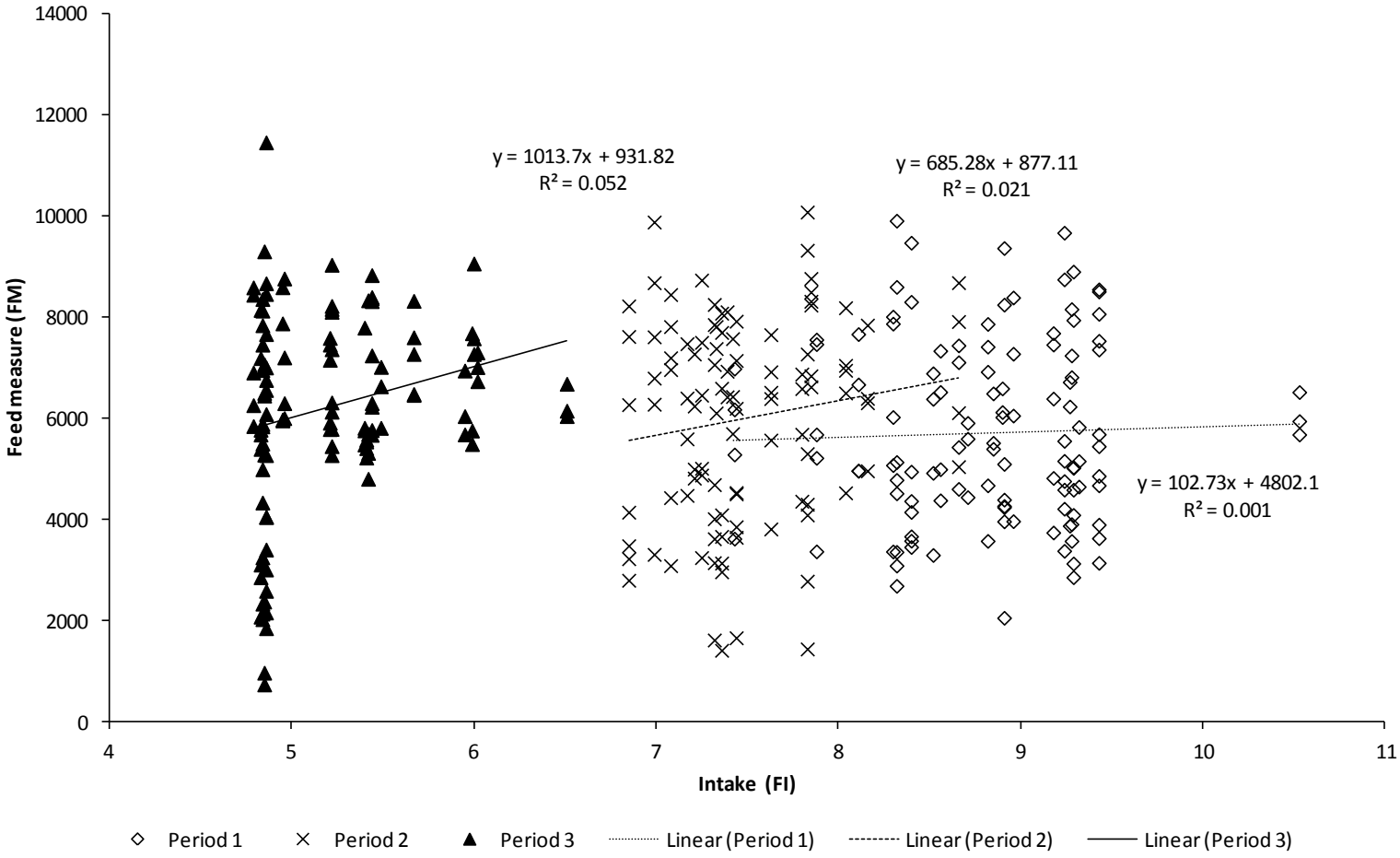


Figure 3.5.16 Linear regression analysis of Feed Measure FM on feed intake expressed as % of body weight, pooled across feeding treatments.

3.6 Trial 6– Measurement of visceral warming patterns in commercially grown southern bluefin tuna (*Thunnus maccoyii*) in response to two feeding regimes

Data from Trial 5 suggests that feed intake cannot be predicted through interpretation of visceral warming patterns. With higher water temperatures, feed intake will be higher and the mean feed measure will be lower. With cooler water temperatures, feed intake will be lower and mean feed measure values will be higher suggesting heat is expended in warm water and conserved in cool water. It does not appear that visceral warming is influenced by dietary energy when SBT are fed two times per day, although one feeding regime showed differences that were not necessarily related to dietary energy intake.

In Trial 6, 15 DST-Centi archival tags (Star-Oddi, Iceland) were inserted into each SBT feeding regime group. Mortalities were experienced in both groups and it is suspected that this was due to the tagging process. Four tags were deployed in SBT from *Sekol Farmed Tuna* and three tags were deployed in SBT from *Kistuna* but did not yield reliable data. Tag data showed that the SBT did not fully recover from surgery and feeding events were limited. As a result these tags were excluded from the analysis.

Seven SBT ranched by *Sekol Farmed Tuna* yielded 18 recordings each (six in each sampling Period) and total of 106 records were considered in this analysis. Data were collected from nine SBT ranched by *Kistuna*, each SBT yielding 18 records. A total of 150 records were considered for this group. For *Sekol Farmed Tuna*, the number of feeding events ranged from none to two per day. For *Kistuna*, the number of feeding events ranged from one to six per day. However, there were times when the SBT consumed either fewer or more meals than offered. The number of consumed meals in the individual data for *Sekol Farmed Tuna* ranged from none to three (Figure 3.6.1), and for *Kistuna*, from none to six per day (Figure 3.6.2). A feeding event is characterised in visceral warming patterns by a sharp drop in visceral temperature (T_{vis}) as a result of ingesting food that is the same temperature of water or cooler.

The water temperature (T_w) followed a similar pattern to other years in the tuna farming offshore zone: starting at approximately 23^{o}C in early March and dropping to 14^{o}C by mid winter (Figure 3.6.3).

The mean values for the variables in this Trial are listed in Table 3.6.1. For *Sekol Farmed Tuna*, the mean number of feeding events was 1.28 meals per day, while for *Kistuna* it was 4.11 meals per day. For *Sekol Farmed Tuna*, there were 22 instances in which the SBT consumed more meals than they were offered. This is likely due to incorrect recording of feeding times by the company. It is also possible that baitfish swam into the pontoon and was consumed by the SBT as evidenced in Figure 3.6.2 where the SBT consumed a meal just before midnight.

For *Kistuna* there were only two events when the SBT ate an additional meal to what was offered. It should also be noted that in Period 2, fewer meals were offered and consumed by SBT ranched by both companies and the FM values were also lower in this Period.

Table 3.6.1 Mean values (±SE) for variables used in this study. Period 1: 19-24 April; Period 2: 20-25 May; Period 3: 21-26 June.

Company	Feeding Events	Meals Eaten	Feed Measure
Sekol Farmed Tuna	1.28 ±0.07	1.41 ±0.09	5385.68 ±149.56
- ***Period 1***	1.67 ±0.07	1.77 ±0.16	6118.61 ±180.20
- ***Period 2***	0.83 ±0.11	0.97 ±0.13	4497.97 ±224.72
- ***Period 3***	1.33 ±0.12	1.58 ±0.13	5573.84 ±257.92
Kistuna	4.11 ±0.11	2.75 ±0.11	5016.10 ±159.08
- ***Period 1***	4.33 ±0.21	3.33 ±0.21	5335.10 ±200.58
- ***Period 2***	3.50 ±0.11	2.31 ±0.13	4097.64 ±237.76
- ***Period 3***	4.50 ±0.24	2.54 ±0.17	5563.21 ±338.22

Differences in feed measure according to number of feeds, company, and Period

Separate GLM analyses were conducted to establish the factors involved in predicting FM.

Period

There were significant differences in the pooled FM data according to the Period when data were collected ($F = 16.406$, $df = 2, 227$, $p < 0.001$). Post hoc testing revealed that Period 2 was significantly lower ($p < 0.001$) than either Periods 1 or 3, which did not differ from each other (Figure 3.8.1).

Company

The grand FM means for *Sekol Farmed Tuna* and *Kistuna* were not statistically different ($F = 2.350$, $df = 1, 228$, $p = 0.127$).

Period by company

Analysis of a possible interaction by company and Period showed effects of Period ($F = 15.710$, $df = 2, 224$, $p < 0.001$), marginal effects of company ($F = 3.088$, $df = 1, 224$, $p = 0.080$), and no interaction between Period and company ($p = 0.377$). Overall, these data support the individual analyses: the FM for *Sekol Farmed Tuna* and *Kistuna* did not differ significantly but Period 2 had lower FM values than either Periods 1 or 3 for both companies (Figure 3.8.4). Given the absence of a Period by company interaction, the effect of Period was not considered further and data were pooled to facilitate company comparisons.

Number of feeding events

The relationship between FM and the number of feeding events for each company is shown in Figure 3.6.5. The relationship showed a relatively linear pattern for *Kistuna*, with FM increasing proportionally to the number of feeding events: $Y = 494.86 + 2978.2$, $R^2 = 0.835$. For *Sekol Farmed Tuna* the mean FM values were higher both at one ($p = 0.027$) and two ($p < 0.001$) feeding events than they were for *Kistuna*, as there were only one or two feeding events in this company.

The relationship between FM and number of feeds for *Sekol Farmed Tuna* was: *Y = 1037.2x + 3792.* GLM analysis of the effects of company, number of feeding events, and their interaction on FM indicated a significant effect of feeding events ($F = 17.592$, df = 1, 226, $p < 0.001$), but not of company ($p = 0.201$) or their interaction ($p = 0.161$). Therefore, FM increased with feeding events, but when considering the total amount consumed across all feeds, the companies did not differ.

Number of feeds consumed

This analysis examined the number of feeds actually consumed (Figure 3.6.6), not what was offered. FM increased according to the number of feeds consumed for both companies. For *Sekol Farmed Tuna*, a very strong linear relationship was observed: $y = 894.42x + 3711.6$, $R^2 = 0.994$. For *Kistuna*, the relationship between FM and number of feeds consumed was relatively linear up to five feeds per day, and dropped off somewhat at 6 feeds per day. The linear equation for *Kistuna* was $y = 489.53x + 3572.3$, $R^2 = 0.788$. However, a cubic relationship provided a much closer approximation to the data: $y = -87.725x^3 + 795.82x^2 – 1414.3x + 4613.5$, $R^2 = 0.992$.

GLM analysis of the effects of company, number of meals consumed, and their interaction on FM mirrored the results obtained for the number of feeds offered. There were significant effects of feeds consumed ($F = 17.938$, df = 1, 226, $p < 0.001$) but not of company ($p = 0.223$) or their interaction ($p = 0.747$). Therefore, the FM increased according to the number of meals consumed, but the results for the two companies did not differ from one another overall.

Difference between feeds offered and consumed

SBT often ate fewer or more meals than they were offered as shown in Table 3.6.2. There were a higher proportion of *Sekol Farmed Tuna* that consumed more meals than they were offered: there were 22 instances (20.7%) where the meals consumed exceeded the meals offered. For *Kistuna*, there were only two instances (1.3%) where the SBT ate more frequently than offered. In contrast, there were many instances where *Kistuna* SBT ate fewer meals than offered, which is likely to be explained by the higher number of meals offered by this company.

Table 3.6.2 Difference between meals offered and meals consumed for *Sekol Farmed Tuna* and *Kistuna* in Trial 6.

Meals Offered – Meals Consumed	*Sekol Farmed Tuna*	*Kistuna*
-2	2	0
-1	20	2
0	77	43
1	7	42
2	-	33
3	-	22
4	-	7
5	-	1
Total	106	150

The mean FM according to the difference in feeds offered and consumed are shown in Figure 3.6.4. GLM analysis revealed no significant effects of the number of feeds offered and eaten ($p = 0.808$), the company ($p = 0.220$), or the interaction ($p = 0.734$). Although there was great fluctuation in the means, there appeared to be trends in both companies for the FM values to decrease as the difference between feeds offered and consumed increased. In other words, the fewer feeds were consumed (in relation to what were offered), the lower the FM appeared to be (Figure 3.6.6).

Data were classified into three groups according to whether the SBT (a) ate more than offered, (b) ate the number of feeds offered, or (c) ate less than offered. These results are shown in Figure 3.6.7. No clear pattern emerged between or within the companies.

Summary

SBT consumed more meals at *Sekol Farmed Tuna* than they were offered (22 instances or 20.7% where the meals consumed exceeded the meals offered) but this result is likely to be due to incorrect recording of feeding times by the company. There were only two instances (1.3%) where *Kistuna* SBT ate more frequently than offered.

There were significant differences in the pooled FM data according to the Period when data were collected. Period 2 was significantly different than either Period 1 or 3.

The grand FM means for *Sekol Farmed Tuna* and *Kistuna* tuna were not statistically different. However, closer examination showed that it took *Kistuna* five feeds to meet the same FM mean score that was achieved by *Sekol Farmed Tuna* with two feeds. Therefore, FM increased with feeding events, but when considering the total amount consumed across all feeds, the companies did not differ from one another.

Data were classified into three groups according to whether the SBT (a) ate more than offered, (b) ate the number of feeds offered, or (c) ate less than offered. These results are shown in Figure 3.6.7. No clear pattern emerged between or within the companies. The number of feeds in *Sekol Farmed Tuna* was significantly less than the number of feeds in *Kistuna*. In both companies, the FM increased in relation to the number of feeds offered or consumed. However, the FM values overall did not differ between the companies.

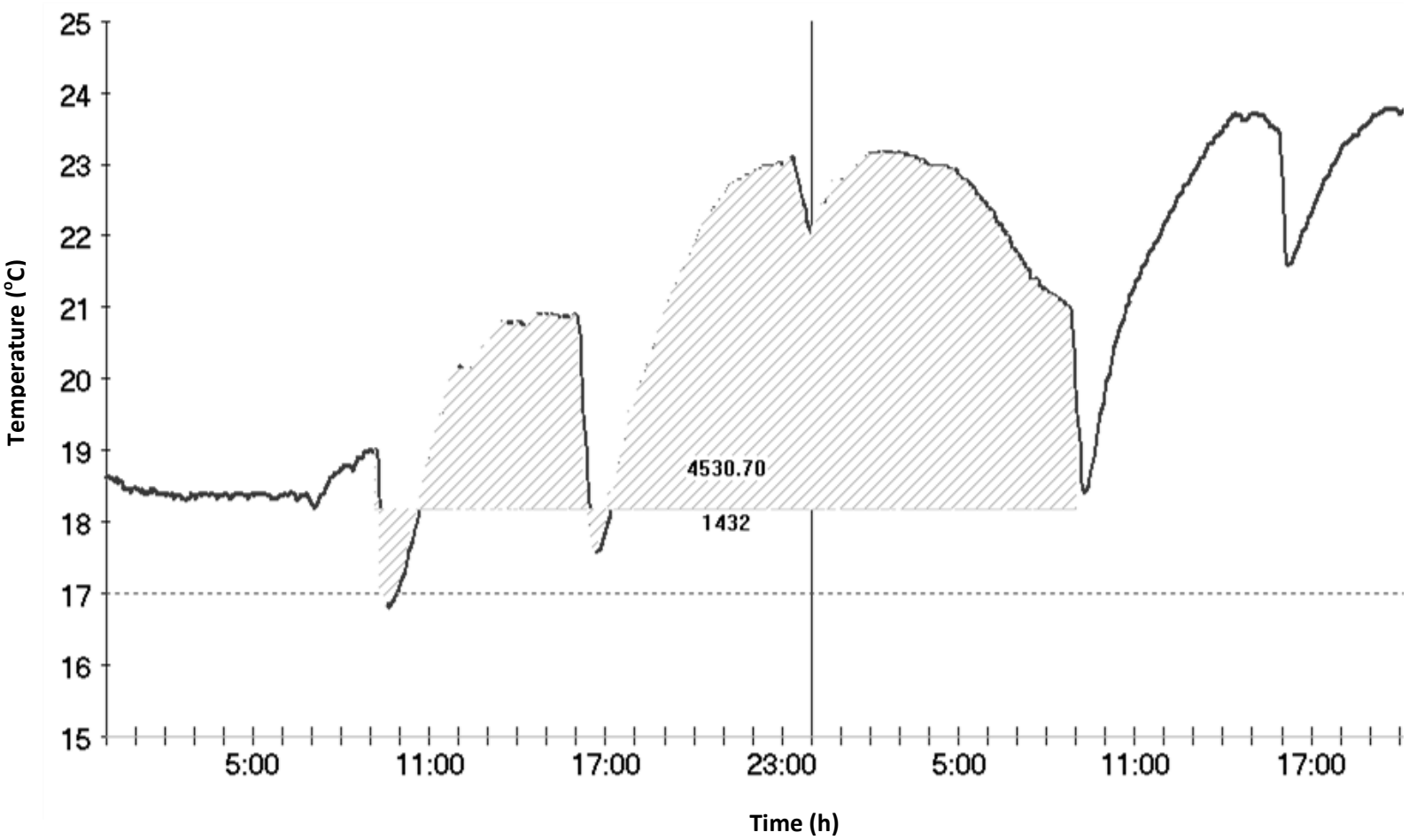


Figure 3.6.1 SBT visceral warming pattern expressed as a function of temperature area under the curve (FI) in relation to time (h) in response to two meals and a 'midnight snack'.

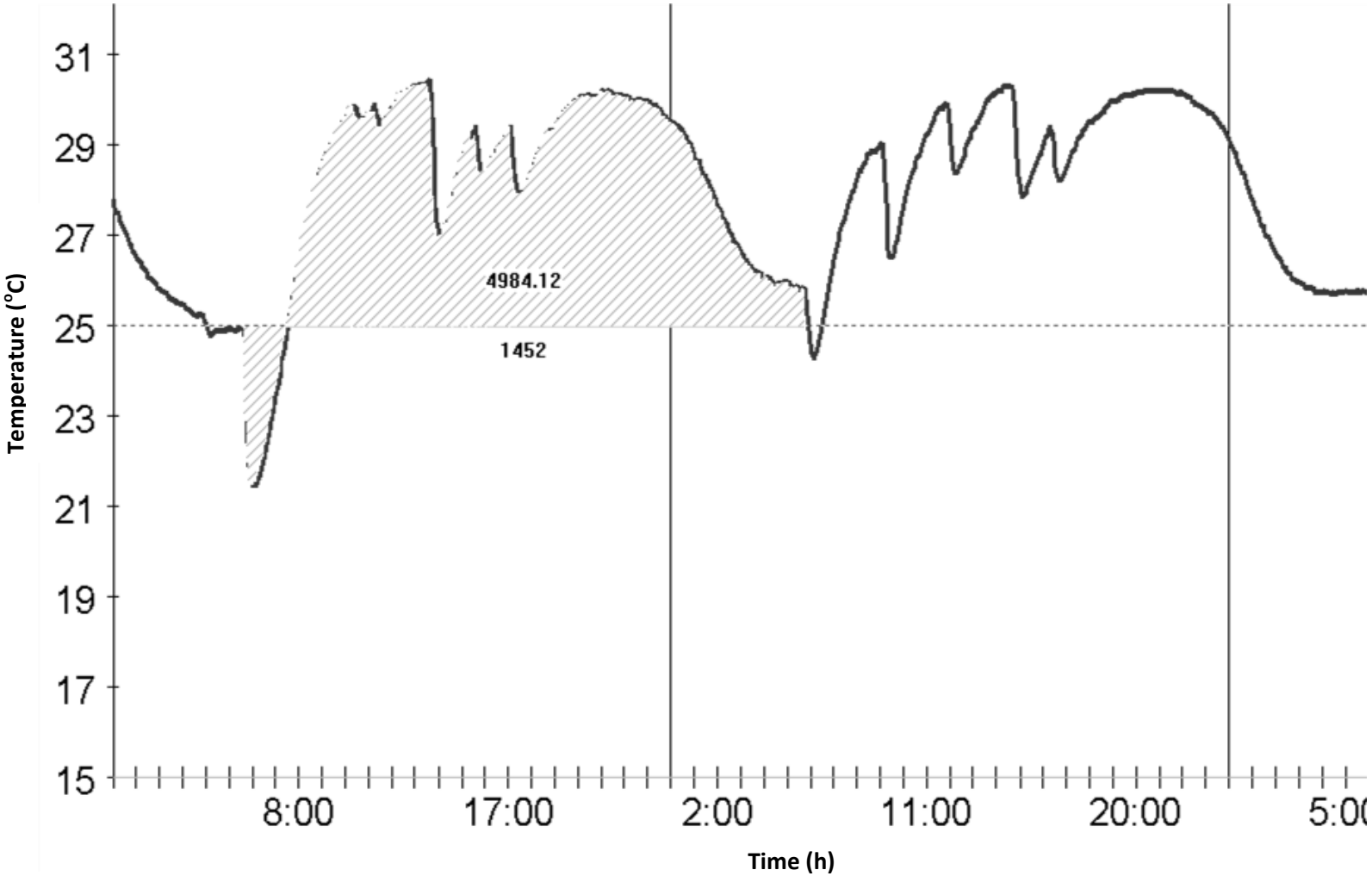


Figure 3.6.2 SBT visceral warming pattern expressed as a function of temperature area under the curve (FI) in relation to time (h) in response to six meals.

Figure 3.6.3 Mean daily water temperature (^{o}C) during the experimental Period (date) measured at a depth of 5m using a Vemco data recorder.

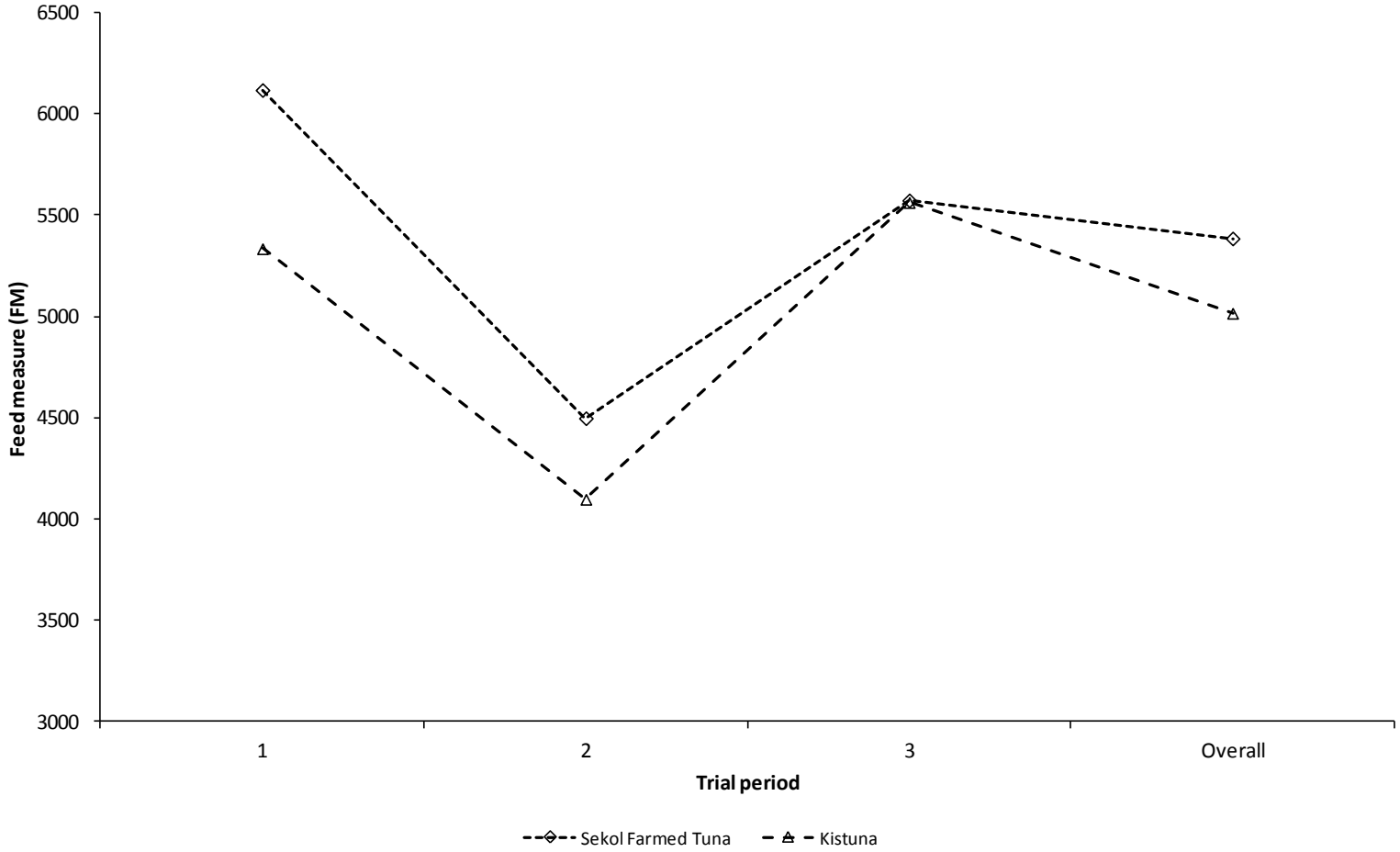


Figure 3.6.4 The relationship of mean feed measure (FM) for each recording time Period and overall for *Sekol Farmed Tuna* and *Kistuna* ranched tuna.

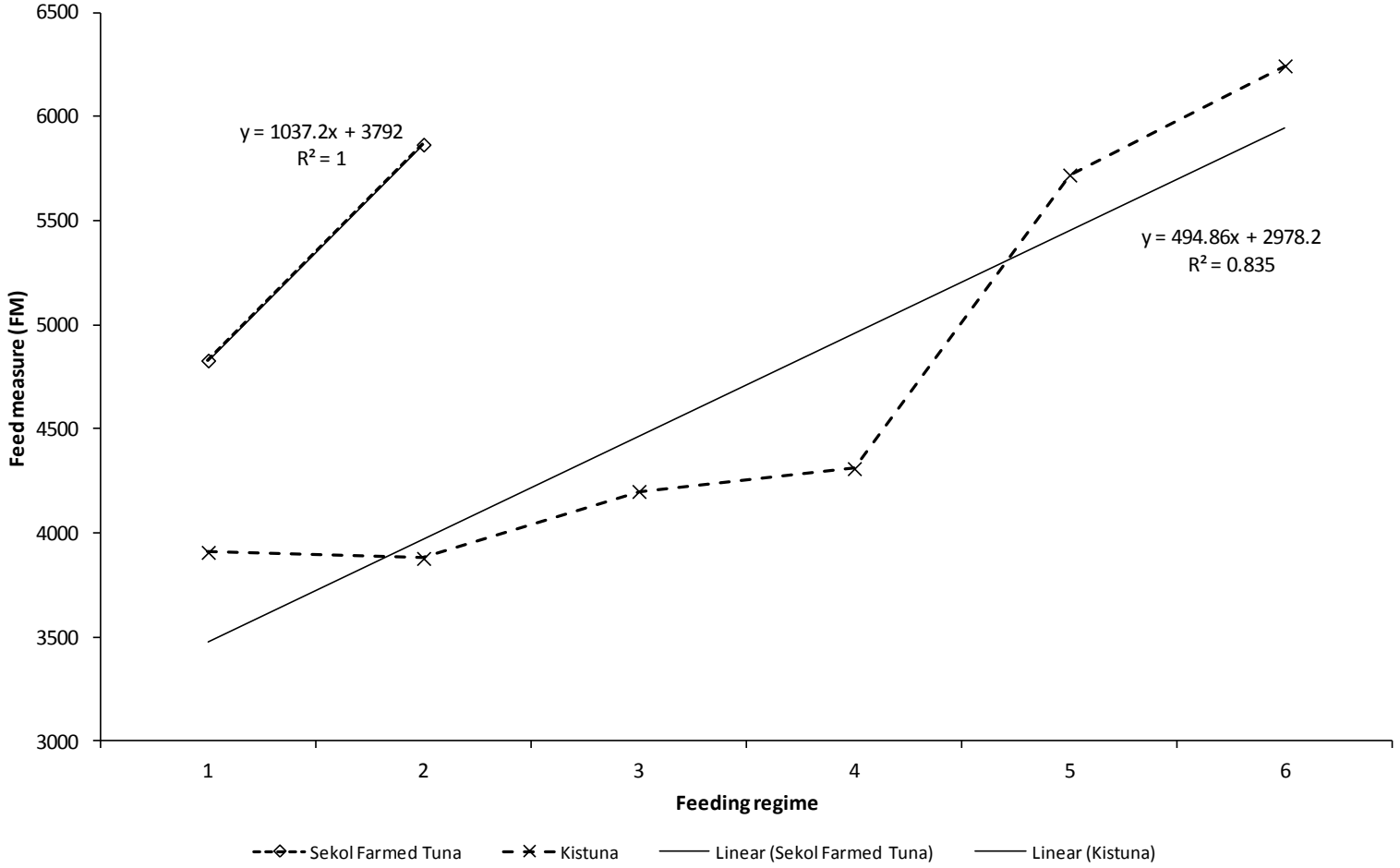


Figure 3.6.5 The relationship between mean feed measure (FM) and feeding events for *Sekol Farmed Tuna* and *Kistuna* ranched tuna. General linear regression lines are shown as smoothed lines for each company.

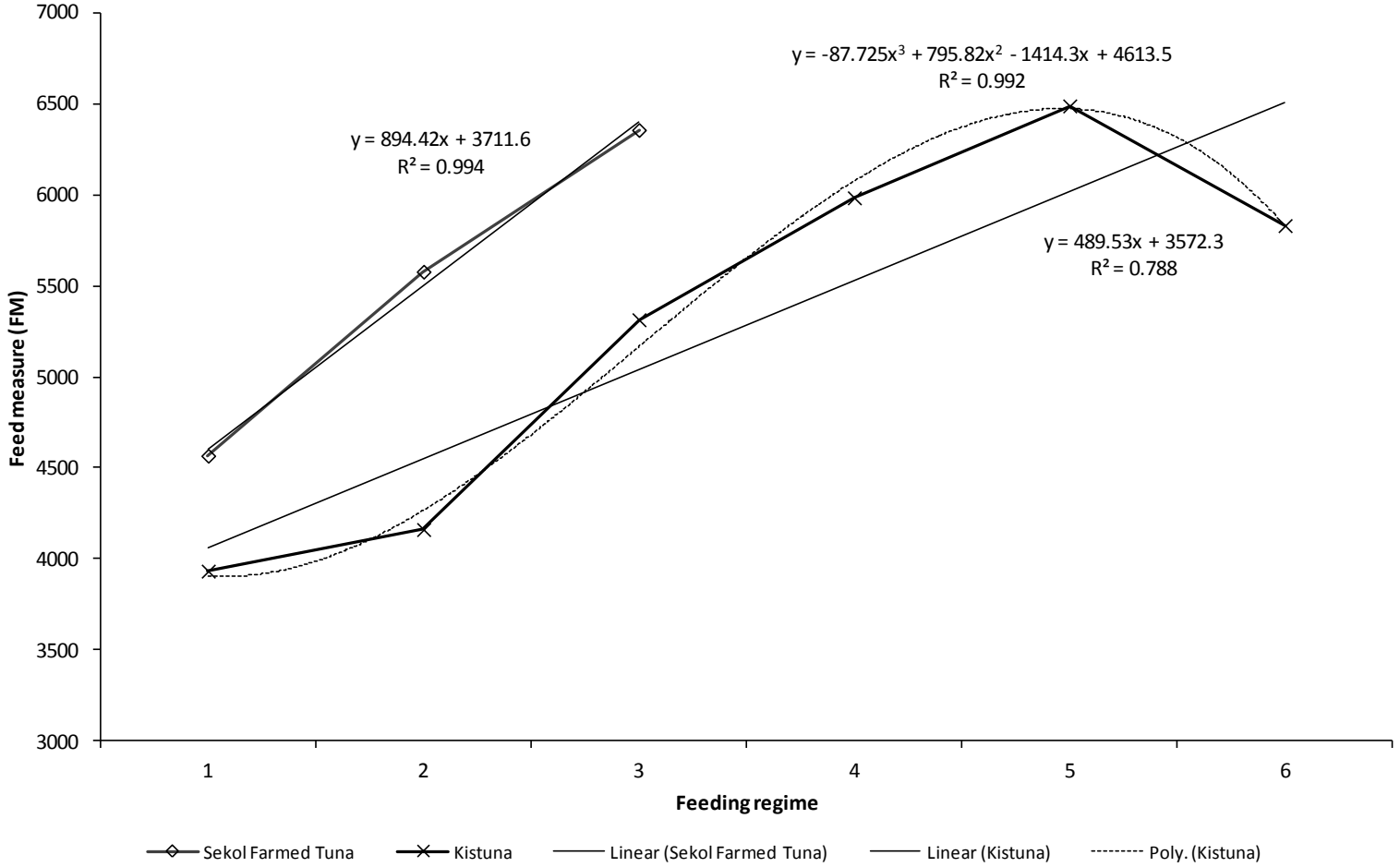


Figure 3.6.6 The relationship between mean feed measure (FM) and feeding events for *Sekol Farmed Tuna* and *Kistuna* ranched tuna. General linear regression lines are shown as smoothed lines for each company. Polynomial regression line is shown for *Kistuna*.

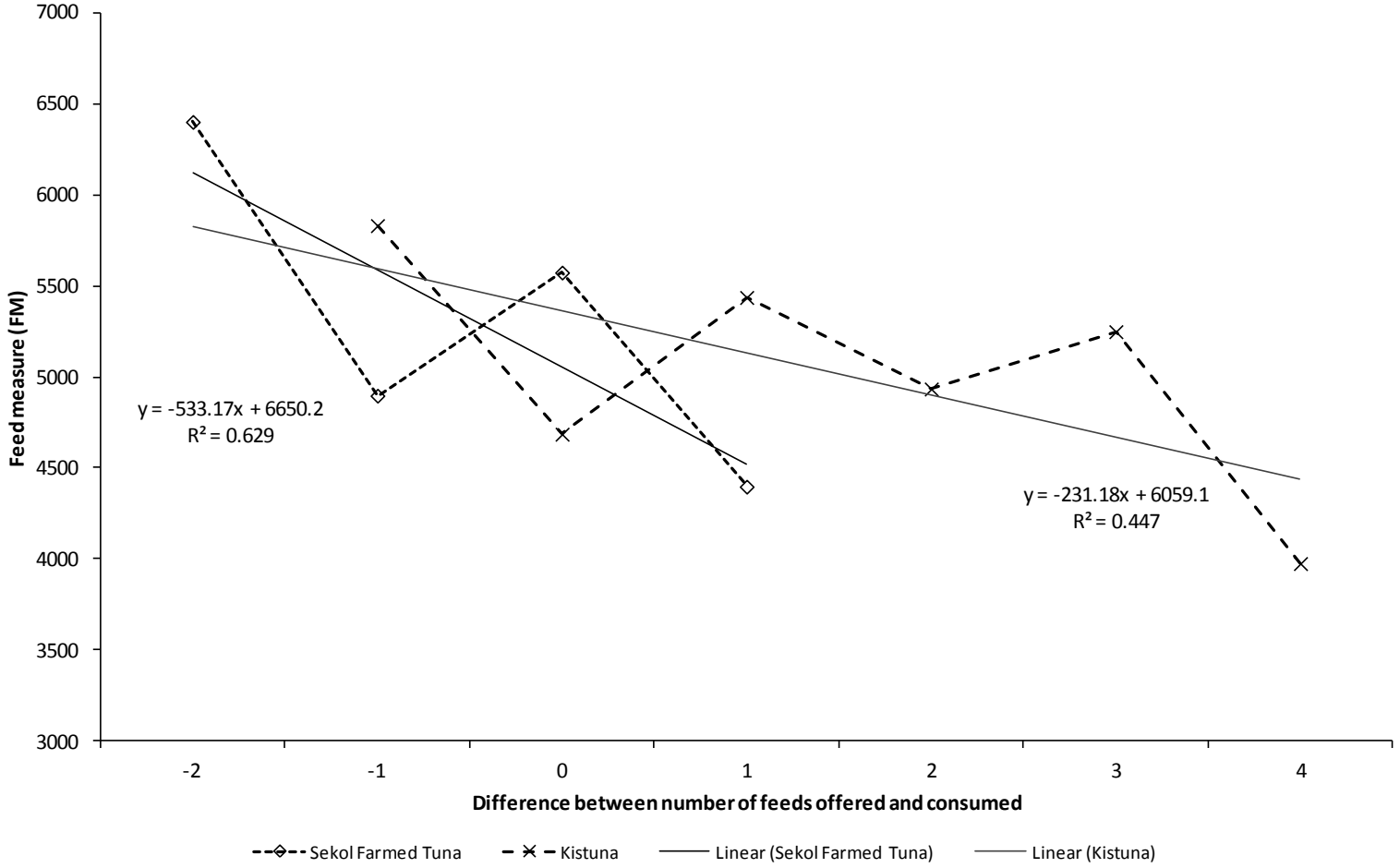


Figure 3.6.7 The relationship of mean FM in response to feeds offered and consumed. Negative numbers indicate that more meals were consumed than offered, while positive numbers indicate that fewer meals were consumed than offered. Linear regression lines for each company are also shown as smoothed lines.

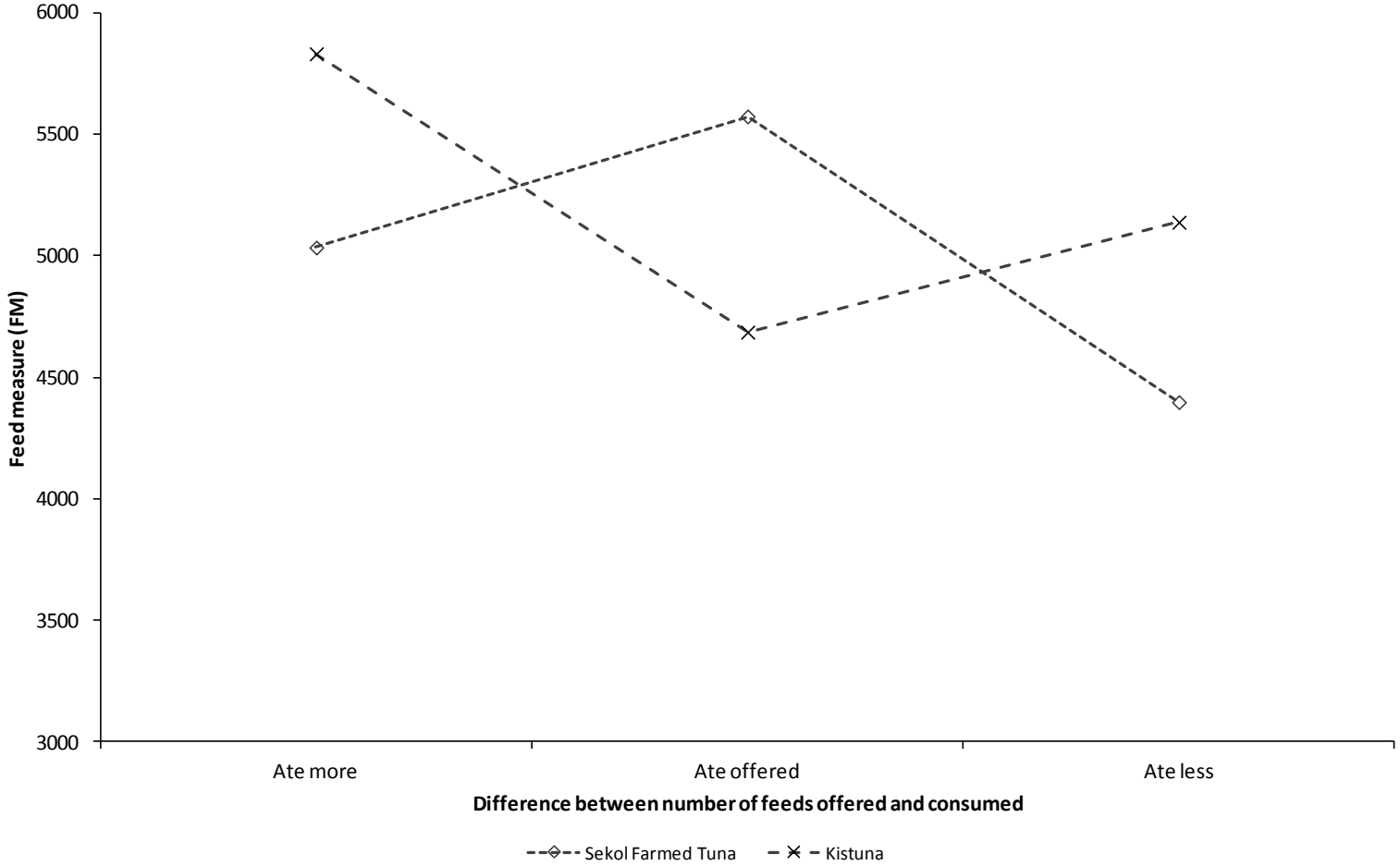


Figure 3.6.8 The mean FM response to when SBT ate more, what was offered or less for *Sekol Farmed Tuna* and *Kistuna* ranched tuna.

Chapter 4 - Discussion

This section presents a discussion on the results of the six trials and describes factors that were found to influence rate, amount and duration of visceral warming in SBT. Regional endothermy and the relationship between feeding and ambient water temperature is described and industry feeding practices and feeding frequencies are considered with regard to SBT health and ranching productivity.

4.1 General Trial limitations

It is accepted that tuna in general, are difficult to work with (Korsmeyer and Dewar, 2001; Clark et al., 2008) and it is a challenge to develop robust experiments that optimise statistical power. To increase statistical confidence in results requires cage/pontoon replication if small numbers of SBT are stocked or the number of pontoons can be reduced if the sample size is increased (Haskard et al., 2001). Both of these scenarios have the potential to introduce significant costs to research and stretch limited resources.

This thesis has used SBT as independent experimental units and it is considered as pseudo-replication and can result in over estimating or overstating the significance of a result as true randomness is difficult to achieve (Haskard et al., 2001; Millar and Anderson, 2004).

To reduce the impact of pseudo-replication and ensure research methods reflect commercial conditions, SBT were stocked at a similar density in tow pontoons and static ranching pontoons, equipment, processes and sites were as consistent as possible with commercial infrastructure, and ranching conditions were as close as possible to commercial conditions (Riley and Edwards, 1998; Haskard et al., 2001).

Generally if the same subjects are repeated, a different type of analysis (i.e. within-subjects/paired type tests rather than between-subjects) can be undertaken. Between-subjects analyses assume that the subjects are independent. The subjects in the thermodynamic analysis may have correlated error terms, which is a violation of the independence assumptions. Often the same SBT on different days are making up a sample at any point in time.

Ideally, the measurements would all come from different SBT. Furthermore, there are comparisons across time (e.g. Period 1, 2 and 3) in which data from some of the SBT are repeated. These are treated as if they are independent groups, when in reality, it is an overlapping group measured more than once. If all independent SBT were measured only once at each time Period, then a within-subjects analysis could have been undertaken, however this is not easily achievable when working with SBT.

An important issue that was considered as part of the current research was the potential impacts of physiological stressors on SBT as a result of handling through capture using baited, barbless hooks and handlines, air exposure, measurement cradles, stocking research pontoons through direct handling, the insertion of conventional dart tags and invasive surgery to implant archival tags. Gunn et al. (2002) and Glencross et al. (2002) have highlighted this issue in their analyses.

Studies on the effect of catch and release angling conducted on largemouth bass (*M. salmoides*) shows that this species is not affected by this practice (Quinn, 1989; Pope and Wilde, 2004), however studies suggest it can impact on physiological processes including impacts on short term health and condition (Cooke et al., 2000; Cooke et al., 2002) Rainbow trout (*Oncorhynchus mykiss)* (Pope et al., 2007) have also been found to be resilient to catch and release. However some researchers have suggested that frequent capture and handling of large and smallmouth bass could have an impact on behaviour (Keiffer et al., 1995; Philipp et al., 1997). Other studies have shown impacts of post release survival on larger fish (Loftus et al., 1988; Schisler and Bergersen, 1996, Stokesbury et al., 2011). Ultimately different species of fish respond in different ways to handling depending on the degree of intensity, inflicted injuries, frequency, age, number of captures, methods of handling and equipment used (McLeay et al., 2002).

Handling and tagging of fish is recognised as having an impact on their survival and growth (Crozier and Kennedy, 2002; Sumpton et al., 2008). As all SBT in this trial were tagged, the results were interpreted as comparisons between feeding regimes rather than absolute values, as the extent of the tagging impact could not be measured.

To minimise errors associated with measurement of feed intake, it has been suggested that a single fish should be placed in a tank for monitoring (Ranta and Pirhonen, 2006). However

there are a number of problems associated with this approach: should the fish die, the observational unit is lost, single fish do not acclimatise well in tanks, and maintaining fish for 12 – 16 months leads to problems especially when performing diet manipulations which may have led to mortalities (Kousoulaki et al., 2007; Videl et al., 2008). Scoping studies initiated for this research in 2004 tested the potential to hold individual SBT in a pontoon but all fish in these experiments refused to eat and died.

4.2 Trial 1 – The visceral warming response in southern bluefin tuna (*Thunnus maccoyii*) to a single meal with an emphasis on volume of feed ingested, dietary energy and baitfish size

The pattern of visceral warming exhibited by SBT in this study was consistent with previous research (Carey et al., 1984; Gunn et al., 2002). Visceral heat is produced by the hydrolytic breakdown of food consumed and metabolic activity is thought to aid in digestion and is restricted to digestive processes (Clark et al., 2008). The heat is then retained in the body due to the insulation capacity of the visceral cavity (Gunn et al., 2002) and the advanced visceral rete counter current heat exchange system found in tunas (Fudge and Stevens, 1996) that supplies blood to the spleen, stomach, caecum and intestines. Blood supply to the liver and white muscle does not pass through retia mirabilia and they should not accumulate heat (Carey et al., 1984; Fudge and Stevens, 1996). However, it has been hypothesised that the liver retains heat through the ventral surfaces that have distinctive patterns of radiating vessels, which resemble two dimensional heat exchangers (Carey et al., 1984; Fudge and Stevens, 1996). Although the functions of these vessels has not been determined, temperature measurements of livers from freshly killed Atlantic bluefin displayed a proximal/distal temperature gradient (Carey et al., 1984) and it is therefore possible that the liver acts as insulation between the viscera and the cold heart, kidney and gills.

The first stage of visceral warming is related to the hydrolytic breakdown of food in the stomach until it reaches a maximum temperature (t_{max}). Stomach contractions occur at t_{max} and it is believed that this results in forcing the digesta into the intestines (Carey et al., 1984). Following on from the emptying of the stomach it is assumed that hydrolysis

continues in the caecum and that the temperature of the visceral cavity returns to basal temperature (T_b) at the completion of digestion (Carey et al., 1984; Carter et al., 1999; Gunn et al., 2002).

Feed Indicator and diet energy

Tuna have a very efficient digestive system that allows them to feed frequently and digest food efficiently and rapidly when food is abundant (Carey et al., 1984). Gastric evacuation in tuna (≈10 – 12 h) has been reported to be significantly faster than that of other teleosts (Magnuson, 1969; Schaeffer, 1984; Olson and Boggs, 1986; Brill, 1996). Furthermore, visceral temperatures can remain elevated for up to 48 h after feeding (Figure 3.1.2) and is considered to be a post-absorptive metabolic effect attributed to anabolic activities such as protein synthesis (Brown and Cameron, 1991a; Brown and Cameron, 1991b; Carter and Houlihan, 2001) or catabolism events like amino acid deamination (Beamish and Trippel, 1990).

Visceral warming patterns (Figure 3.1.2 and Figure 3.1.5) demonstrate visceral warming responses to a 1 kg ration of varying lipid content but relatively consistent protein content (Table 3.1.1). It appears that visceral warming is not solely attributed to anabolic or catabolic processes associated with protein but non-protein energy sources impact on visceral warming. The data presented in this research suggests there was a significant mean difference in FM between bait types and visceral warming used in this study (Figure 3.1.6). Furthermore, *S. sagax* (US) was significantly different to other baitfish used in this analysis (Figure 3.1.8) which may be associated with the size of the baitfish but would more likely be associated with the energy content of the feed.

When all baitfish types were pooled the relationship was better modelled by a non-linear relationship which may reflect how SBT regulate visceral warming in response to energy content of the feed. It appears that the visceral temperature increases to a certain point above basal and then plateaus (Figure 3.1.9). It is believed that large bluefin tuna need to live in cooler waters to avoid overheating (Kitagawa, et al., 2006) and a regulatory mechanism that limits visceral temperature would achieve this.

Energy and duration of visceral warming

Bait type did not affect the duration of visceral warming whereas dietary energy had a significant influence. This would appear to reflect post-absorptive metabolism and be explained by up-regulation due to increased nutrient assimilation. Given that gastric evacuation in tuna (≈10 – 12 h) has been reported to be significantly faster than that of other fish (Magnuson, 1969; Schaeffer, 1984; Olson and Boggs, 1986; Brill, 1996), and anecdotally, after SBT have been fed the evening before there is no feed in their stomachs the following morning during harvest activities, that dietary energy does influence the duration of visceral warming.

Ultimately the results of this trial suggest SBT are managing the assimilation of nutrients and consequent visceral warming to meet metabolism and physiology. Given their physiological requirements and the vast distances they travel in the nutrient deficient epi-pelagic layer of the world's ocean (Caton, 1991) it is plausible that SBT would optimise the use of food in different thermal habitats.

t_{max} and feed intake

Gunn et al (2002) found a very strong linear relationship between t_{max} and food intake for fish in winter and summer, and a strong separation between fish in winter and summer (i.e. they produced different slopes). Similarly, they found very little difference in maximum heat increment and food intake with little difference between sizes of SBT in winter. Whilst these small differences could be partially and possibly explained by tag position or biological variation, the key factor missing in their trial design and assessment was the interaction of energy in the baitfish and water temperature (T_w) on t_{max}.

This Trial demonstrated a statistically significant relationship between t_{max} and dietary energy where the higher the energy content of the feed consumed led to a stronger linear relationship with increasing energy. Furthermore, dietary energy influences the time taken to reach t_{max}.

Trial results (Figures 3.1.2 – 3.1.5) show that a diet high in energy takes up to four times longer than a diet low in energy suggesting that there is some mechanism detecting the nutritional status of feed which in turn influences the rate and duration of visceral warming. The rate of visceral warming is also likely to be linked to nutritional content of the ingested feed.

There was a significant difference between bait types and t_{max}. The strong linear regression significantly changed with the energy content of the feed as opposed to size of baitfish, suggesting that energy is the driving factor behind t_{max}, although baitfish size cannot be totally ruled out.

Ration (baitfish) size and visceral warming

It has been reported that GET is related to peak visceral warming (Carey et al., 1984; Gunn et al., 2002) and this research has demonstrated significant differences in the slopes of t_{max} in response to dietary energy. Specifically, t_{max} was reached in approximately 30 h when 1 kg of high energy feed was fed to SBT. The mean baitfish ration sizes used in this Trial were – *S. sagax* (US) – 100 g, *S. pilchardus* – 50 g, *S. sagax* (EC) 38 g and *S. sagax* (PL) – 27 g (Table 3.1.1) and it is postulated that baitfish size does affect t_{max} and visceral warming to some degree.

Research has demonstrated that manufactured diet pellet size does not impact on growth in Atlantic salmon (*S. salar*) (Bailey et al., 2003). Similarly no difference in manufactured diet pellet acceptance has been shown in a study involving three different size manufactured diet pellets fed to the same size Atlantic halibut (*Hippoglossus hippoglossus*). However the study group that received the three manufactured diet pellet sizes grew better than the group that received two manufactured diet pellets sizes (Helland et al., 1997). The findings relate more to whether intake can be increased by providing a smaller manufactured diet pellet size in addition to larger size manufactured diet pellets. Whether this relates to gut evacuation rate is unclear. However, it has been demonstrated in a feed trial involving different size manufactured diet pellets in Nile tilapia (*Oreochromis niloticus*) that growth heterogeneity is proportional to food particle size and food intake and gastric evacuation rates are inversely proportional to food particle size (Azaza et al., 2010).

The results of this study showed that the difference in the baitfish types cannot be ruled out as not having an influence in the time taken to reach t_{max} as the mean size of *S. sagax* (US) is twice that of *S. pilchardus*. However, given that *S. sagax* (EC) was smaller in size than *S. pilchardus* and produced a linear relationship that reflected a higher energy content suggests it is unlikely that bait size is having a significant effect.

Anticipatory feeding behaviour

Observations of visceral warming patterns in this trial indicated anticipatory feeding behaviour. SBT were found to often elevate their visceral temperature in anticipation of being fed (Figure 3.1.2), and have been shown to swim faster after consuming a meal (Fitzgibbon et al., 2007). Tunas are often referred to as energy speculators investing high amounts of energy in relation to expected returns (Stevens and Neill, 1978; Fitzgibbon et al., 2007).

In this trial, there was evidence of feeding anticipation whereby the SBT to be fed would start to increase their visceral temperature above basal temperature at the approximate time of their scheduled feed (Figure 3.1.2). In this situation, the SBT appeared to anticipate a meal at 9 am and started to elevate visceral temperature, however they were actually fed six hours later. This behaviour is not unique to SBT but observed in many species of farmed fish where anticipatory feeding behaviour is linked to circadian rhythms (Spieler, 1992), light and entrainment (Sanchez-Vazquez and Madrid, 2001), and restricted feeding (Purser and Chen, 2001) and is believed to be associated with a large release of cholinergic (vagal) inhibition (Keen et al., 1995).

Clark et al. (2008) found that SBT would increase their heart rate in addition to warming their viscera. This is consistent with other species that can be trained to accept feed at pre-defined times (Chen and Tabata, 2002; Brännäs et al., 2005; Vera et al., 2007) and that this has a likely energetic cost. Furthermore, anticipatory feeding behaviour has been demonstrated in greenback flounder (*Rhombosolea tapirina*) and it has been hypothesised that this species is capable of evaluating the energetic and temporal impacts of a single daily meal (Purser and Chen, 2001).

Anticipatory physiological regulation is an adaptive strategy that allows animals to respond faster to physiologic and metabolic challenges and this anticipatory response prepares animals to digest, absorb and metabolise nutrients thereby enhancing fat absorption and links digestion and metabolism (Power and Schulkin, 2008). This further supports the finding that the extended visceral warming response is related to Periods when SBT are fed high energy diets.

Summary

This trial demonstrated that SBT have a physiology that enables them to manage visceral warming independent of the nutrient content of their feed, and that:

1. There is a strong relationship between FM and diet energy when SBT have been fed a single ration up to 1kg at a mean water temperature of 14.7^{o}C. Where the nutritional content of feed is known, intake may be predicted with measurable certainty.
2. Dietary energy has a significant impact on the duration of visceral warming at a mean water temperature of 14.7^{o}C and is not necessarily linked to the anabolic or catabolic pathways of protein synthesis and amino acid deamination.
3. There is a strong relationship between t_{max} and feed intake for each of the baitfish types when SBT have been fed a single ration up to 1kg at a mean water temperature of 14.7^{o}C. Feed intake can be predicted with measurable certainty providing the energy content of the feed is known.
4. Given the evidence of gut evacuation rates in tuna, it is likely that bait size has an influence on visceral warming but needs to be considered in future research.

4.3 Trial 2 – The visceral warming response to one, two or three feeds in southern bluefin tuna (*Thunnus maccoyii*) with an emphasis on weight of feed ingested and dietary energy at two different water temperatures

Previous research using archival tags and measuring visceral warming patterns was undertaken by Gunn et al. (2002) based on feeding a single known ration determined as a percentage of body weight per day ranging from 1 – 12% and measurement of visceral warming responses. Their findings demonstrated that feed intake could be assessed with t_{max} with measurable certainty. However, due to trial limitations there was uncertainty of whether lipid content or food type influenced t_{max}. Trial 1 demonstrated that feed intake can be predicted with measurable certainty using t_{max} and FM providing the feed was a single ration and the energy content of the feed was known. This answered one of the

questions raised by Gunn et al. (2002) and demonstrated that t_{max} is related to the nutritional content and size of ration.

Ration (baitfish) size and nutritional content

There was a slight difference in the size of the baitfish used in this Trial. The mean size of the baitfish used in Period 1 and Period 2 were 53 g and 33 g respectively with a nutritional difference of 3 kJ of energy per 100 g of baitfish fed (Table 3.2.1).

Baitfish size was not considered to have an influence on this trial due to reported tuna gut evacuation rates in tuna of approximately 10 – 12 h (Magnuson, 1969; Schaeffer, 1984; Olson and Boggs, 1986; Brill, 1996) and the outcomes of trial 1 where it was likely that energy was influencing t_{max} as opposed to size of baitfish.

t_{max} and feed intake

As reported in trial 1, t_{max} was influenced by baitfish type and the strength of the linear regression increased with increasing energy content of the feed as opposed to size of baitfish, suggesting that energy is one of the driving factors behind t_{max}.

In this trial, t_{max} was calculated in relation to one, two and three feeds per day during warm and cool water temperatures. The results clearly demonstrate the limitations of using this approach to measure feed intake (Figures 3.2.9 – 3.2.12). One of the challenges in measuring visceral warming patterns was determining basal temperature (T_b) as on occasions it appeared that the viscera hadn't returned to T_b prior to the next meal. To overcome this, T_b was determined as the temperature before the feeding Period started for the week, assuming that T_b is consistent according to water temperature (T_w) (Gunn et al., 2002). Non-return to T_b equates to energy being retained in the system and the likely continued assimilation of nutrients from a previous meal when SBT feed again. As identified in trial 1, dietary energy influences t_{max} and it could therefore be proposed that retained energy within the system of the fish would also have an impact on t_{max}. This was further examined in Trial 4.

t_{max} is recognised as the point where gut evacuation (GET) occurs through the contraction of the stomach (Carey et al., 1984; Gunn et al., 2002). Gunn et al. (2002) reported GET values

based on t_{max} in their study of 588 minutes in summer, and 598 minutes in winter for a 1000 g meal. In this Trial, the mean GET values in Period 1 and Period 2 for a single meal was 423 minutes for a 516 g meal and 696 minutes for a 1140g meal respectively, which is consistent with GET reported in other tuna species (Magnuson, 1969; Schaeffer, 1984; Olson and Boggs, 1986; Brill, 1996).

Given that feeds in this trial were delivered three or six hours apart it is therefore likely that when SBT were fed two or three meals on a given day that GET and therefore t_{max} had not been reached as more food was added to the stomach before the contents were evacuated. This impacted on applying t_{max} as a reliable predictor of feed intake.

Feeding behaviour is another aspect that needs to be considered in the context of t_{max} due to the large variation in daily feed intake that can occur in fish (Jobling et al., 2001). This includes natural feeding behaviour associated with tidal and lunar rhythms as seen in rainbow trout (*O. mykiss*) (Leatherland et al., 1992; Noël and Le Bail, 1997), seasonal shifts in feeding patterns (Smith et al., 1993), and diurnal feeding (Kadri et al., 1997). Social interactions may inhibit feeding even though fish may appear to have fed to excess (Øverli et al., 1998) and environmental conditions can also have direct impact on SBT feeding behaviour (Glencross et al., 2002).

The results of Trial 1 show that if SBT fed to satiation on high energy feed during an evening, feed intake the following day may be impacted. There are limitations in using t_{max} to assess SBT feed intake due to: retained energy in the system; SBT consumption of more than one meal; and inconsistent feeding behaviours. Given these limitations and supported by the findings of this Trial, t_{max} should not be used as a reliable measure of feed intake.

Relationship between feed intake, feeding regime and feed measure

Although Gunn et al. (2002) fed a single ration to determine the relationship between feed intake and FM they found that feed intake and FM changed between seasons. They found that the slopes did not differ in summer yet there was a difference between two groups of fish in winter which they attributed to fish size of less than 27 kg or greater than 31 kg. Despite the differences they found very strong linear and polynomial models when the data were pooled. This is consistent with findings in this current research although there were

differences between intersects and slopes. However, when the data were pooled (Figure 3.2.13) they enabled linear models that allow prediction of intake at two different T_w providing the dietary energy of the feed is known. The measurable certainty in this Trial was greater in cool water than warm water.

Results showed that feed intake was lower during the cooler T_w and higher in warm T_w and was inversely proportional to FM values. In other words, the higher the feed intake the lower the FM and the lower feed intake the higher FM. The data suggests SBT regulate FM as a form of homeostasis in that they expend heat to prevent over-heating when in warm water yet conserve heat when in cooler water. This implies there is an optimum temperature where SBT will not necessarily perform either function providing dietary energy is matched to growing conditions.

Heat conservation in SBT is derived from two features: their thermodynamic capabilities through the use of visceral rete (Fudge and Stevens, 1996) and the insulation of the viscera by the stomach wall, air bladder and surrounding muscles (Gunn et al., 2002). As the T_w warms there is a maximum temperature to which the viscera will warm, beyond which cellular function will be impaired, as is evident in rats (Liu, 2012). To explore this hypothesis a linear regression was used to assess the relationship between T_b and maximum visceral temperature (T_{max}) and T_w.

Relationship between basal visceral temperature, maximum visceral temperature and water temperature - Trials 1 and 2

The T_b and T_{max} data used in this analysis was derived from data from Trials 1 and 2. The data showed that there were significant strong linear relationships for both T_b and T_{max} and that T_b could be predicted by T_w. The range of ambient T_w was approximately 10^{o}C throughout the trial. Research showed that T_b remained 4^{o}C above T_w (Figures 3.2.14 and 3.2.15 whereas previous research demonstrated this differential to be 2^{o}C (Gunn et al., 2002). T_{max} reached above 30^{o}C at approximately 22^{o}C T_w and is consistent with T_{max} values in previous research (Gunn et al., 2002). T_{max} was also not as strongly associated as was T_b with T_w. The reason for this difference is not clear other than it could be attributed to the

placement of the tags or fish condition (i.e. how much fat was in the visceral wall acting as insulation).

There was a break in the data related to the Period between data from Period 1 and Period 2 of Trial 2. This has been used to assess whether there is a structural break in the data where a linear relationship does not exist and where T_w is not a reliable measure of T_b and T_{max}.

The analysis shows that whilst there is a 'flattening' trend in the data it is not significant and that a linear model is the most appropriate analysis based on the range of data at this time.

It has been suggested that tuna warm their viscera to enhance digestion and in laboratory trials using tissues from *T. thynnus* it was assumed that warmer viscera may mean faster digestion rates (Stevens and McLeese, 1984). This is contrary to Gunn et al. (2002) who found that there was no difference in GET and digestion between seasons. The research outcomes in trial 1 of this study demonstrated that GET will vary depending on dietary energy and that dietary energy will influence FM to a point where there will be no more increase in FM.

Gunn et al. (2002) proposed two hypotheses to explain the difference in FM between winter and summer. Put simply, they suggested that GET and digestion remains constant at all T_w and the two reasons for a greater FM in winter are associated with:

1. heat conservation occurring at colder temperatures through changes in the activity of the visceral rete and aerobic metabolism.
2. positive thermal compensation through increased enzyme activity at lower temperatures (Prosser, 1973; Fudge et al., 1997).

Furthermore they suggested that cold activated enzymes are absolutely necessary to increase digestion efficiency allowing wild free roaming SBT to expand their habitats from temperate latitude feeding grounds (10^oC) to the tropical spawning grounds (30^oC).

Whilst these hypotheses are plausible, the data from this Trial suggests there is a homeostatic response whereby SBT expend heat from their system in summer and conserve heat in winter. In large Atlantic bluefin tuna (*T. thynnus*) implanted with archival tags it has been demonstrated that they will dive to depths of 1000 m, frequent areas of water

between 2.8^{o}C and 30.6^{o}C, and maintain a relatively constant internal visceral temperature of approximately 25^{o}C (Block et al., 2001). Yellowfin tuna (*T. albacores*) inhabit warm tropical waters and in 1994 a study found that the same fish made dives very quickly to depths of 300 m where temperatures below the thermocline were 7^{o}C (Block et al., 1997).

The study indicates that this diving behaviour is a response to predators and ship avoidance as opposed to the ability to forage for food in different areas of different T_w (Block et al., 1997).

Another study on yellowfin tuna (*T. albacores*) showed tuna would dive to depths greater than 1000m where the ambient T_w was less than 5^{o}C and tuna would perform repetitive dives to depths of 150 – 250 m to forage below the thermocline (Schaefer et al., 2007). The researchers suggested the tuna could not stay at these depths for very long and had to return to surface layers to warm (Schaefer et al., 2007). It is plausible these dives are a behavioural method to expend heat as a by-product of the digestion process.

In SBT ranching operations, SBT are not able to perform behavioural dives to remove excess heat from their system. They possibly rely on their gills as heat exchangers and perhaps minimise the extent to which they use rete mirabile as counter current heat exchangers (Graham and Dickson, 2001). Similar behaviour has been documented in SBT in a mesocosm respirometer whereby high lipid content sardines induced the greatest postprandial metabolic demand and consequent faster post-feeding swimming behaviour to increase ventilation volume to facilitate oxygen uptake (Fitzgibbon et al., 2007, Fitzgibbon et al., 2008). To increase ventilation SBT must either swim faster or open their mouths wider and flood their gills to absorb as much oxygen as possible and in this situation it is possible that it did serve to deliver oxygen but also allowed heat to be expended from the system. This trial supports the hypothesis that SBT manage visceral warming.

Summary

This Trial demonstrated that:

1. t_{max} is not a reliable tool to measure feed intake in SBT feed based on two meals or more due to retained energy in the system, the combined effect of consumption of two or three meals, and the fact that SBT do not always feed to satiation.

2. There is a stronger relationship between dietary energy and pooled FM responses for all feeding regimes during cooler water temperatures than warm water temperatures.
3. The model developed during cool water temperatures enables prediction of feed intake based on FM providing diet energy is known.
4. There is a homeostasis type adaptation to regional endothermy in SBT and that SBT have a specialised physiological process to manage visceral warming.
5. Cold adapted enzymes possibly have a role in increasing visceral warming during cold water temperatures, as suggested by Gunn et al. (2002) but not proven in this instance.
6. Preliminary data suggests there is an upper threshold limit of visceral warming.

4.4 Trial 3 - The measurement of temperature in red muscle, white muscle and the visceral cavity of slaughtered southern bluefin tuna (*Thunnus maccoyii*) in response to three feeding regimes and ambient water temperature

There has been much discussion over thermoregulation in tunas (Carey et al., 1984; Dizon et al., 1978; Dizon and Brill, 1979a, Dizon and Brill, 1979b; Block et al., 1993; Block, and Finnerty, 1994: Brill et al., 1994; Dickson, 1994; Dickson et al., 2000; Graham and Dickson, 2001; Kitagawa et al., 2001; Dickson and Graham, 2004; Kitagawa et al., 2006). While it is accepted that tunas can regulate temperature through behaviour (such as diving to cooler waters and returning to warm surface waters) (Kitagawa et al., 2004), conjecture still remains on whether they are capable of physiological thermoregulation (Dizon and Brill, 1979b; Brill et al., 1994). In order to demonstrate physiological thermoregulation internal heat retention must be shown to be independent of water temperature (T_w) and heat production (Fitzgibbon et al., 2008).

The results from this Trial demonstrate that SBT maintained red muscle temperature (T_{rm}) at approximately 30^{o}C (29.1 ^{o}C) regardless of feeding schedule or T_w. There was a significant effect of T_w on white muscle temperature (T_{wm}) which was maintained at approximately 6^{o}C above T_w, and the visceral temperature (T_{vis}) was affected by both feeding schedule and T_w,

which was to be expected given the findings in the previous two trials. The 'No feed 24 h' feeding strategy had a good linear relationship with T_w whereas the 'No feed 12 h' did not. This reinforces the view that the SBT viscera needs adequate time to return to basal temperature (T_b) to be predicted providing T_w is known, whereas predicting T_b after 12 h of a feeding event will require the application of the model developed in Trial 2 to predict T_b as shown earlier. The GLM put forward to assess T_b in this Trial reported values higher than those reported in Section 3.2 suggesting additional time is required before T_{vis} returns to T_b, or alternately the capture process has artificially increased T_{vis}.

Muscle temperatures of blue marlin (*Makaira nigricans*) and swordfish (*Xiphias gladius*) were elevated after capture and release (Carey and Robinson, 1981; Block et al., 1992) and Pacific bluefin tuna (*T. orientalis*) fitted with transmitters had elevated muscle temperatures of up to 12^{o}C after handling (Marcinek et al., 2001).

Muscle temperature studies on tuna have shown that acute exercise may increase body temperature (Stevens and Neill, 1978; Dewar et al., 1994; Dickson, 1994). However for exercised ectothermic fishes the maximum increase is only 2.7^{o}C above resting temperature (Dickson, 1994). It has also been shown that large Atlantic bluefin tuna (*T. thynnus*) have lower body temperatures after a prolonged struggle on hook and line (Carey et al., 1984).

Active regulation of temperature in dead fish is unlikely yet heat gain may be possible as a direct result of the harvest process. Capture can impact and significantly affect heat production and retention in tuna (Brill et al., 1994). It is unclear whether the values for T_{rm} and T_{wm} in this Trial are the 'normal' profile of ranched SBT in a resting state. Given temperature recordings were taken as part of commercial ranching harvests, the results were interpreted with caution. Parallel to this Trial, in 2010 wild SBT temperatures were measured using the identical equipment to the current study from identical sites within the Great Australian Bight. From the ten SBT sampled the mean T_{rm} and T_{wm} were 29.3^{o}C and 25.4^{o}C respectively with a 1^{o}C standard deviation taken at a T_w of 19.5^{o}C. These values are consistent with research findings in ranched SBT.

Red muscle, *rete mirabile* length and the number of vessel rows in tuna, increases with age which may improve heat efficiency and conservation (Dickson et al., 2000) and it is believed that large Pacific bluefin tuna (*T. orientalis*) need to inhabit cooler waters to avoid overheating (Kitagawa et al., 2006). This research suggests that SBT are capable of

physiologically thermoregulating their tissues. This research also suggests that commercially ranched SBT that are continually fed maintain T_{rm} at approximately 30^{o}C as the upper threshold or optimum temperature limit.

It has been reported in Atlantic bluefin (*T. thynnus*) that blood supply to the white muscle does not pass through rete mirabilia and therefore it should not accumulate heat (Carey et al., 1984; Fudge and Stevens, 1996). The results from this Trial indicate there is a regulatory process involved in the white muscle. The mean T_{wm} in SBT may be related to the thermal conductance of the red muscle if it is kept in a temperature steady state, and whilst it is a possibility it seems unlikely given the recordings across a T_w range between 14 ^{o}C and 18^{o}C. Alternately, it may be something as basic as the skin and dermal layers acting as a 'wetsuit' thereby creating a heat conservation mechanism.

Visceral warming was a focus of Trials 1 and 2. Larger tuna have the potential for greater thermal inertia due to their thicker viscera walls and more insulating subcutaneous fat (Graham and Dickson, 2001). SBT held at 16^{o}C maintained T_{vis} significantly warmer (0.5^{o}C warmer) at any level of heat production than similar sized fish at 19^{o}C and suggests SBT have an ability to control thermal conductance and are able to physiologically regulate T_{vis} (Fitzgibbon et al., 2008)

There are many examples where tuna have demonstrated a physiologically controlled heat balance (Dizon et al., 1978; Dizon and Brill, 1979a; Dizon and Brill, 1979b). Rete arterial and venule walls have layers of smooth muscles which may provide a mechanism to regulate blood flow (Graham and Dickson, 2001).

This Trial has demonstrated for the first time that there is a relationship between T_{rm} and T_{wm}, feeding and ambient T_w in SBT, and that heat retention is independent of T_w and heat production.

Summary

This trial has demonstrated that:

1. T_{rm} is maintained at 30 ^{o}C regardless of T_w or feeding strategy:
    a. This may present potential problems with the digestion of food as reported in Trials 1 and 2 during Periods of high T_w.

b. SBT have the ability to manage the generation of heat from digestion possibly by both flooding the gills and using them as heat exchanges, as well as managing blood flow through the rete mirabile.

2. SBT maintain T_{wm} at approximately 6 ^{o}C above T_{w} independent of feeding regime
3. T_{vis} is influenced by feeding strategy and T_{w}:
    a. Trials 1 and 2 demonstrated the interaction of feeding and visceral warming.
    b. The interaction between 'No feed 24 h' showed that the viscera will return to T_{b} after time and a relationship exists with T_{w}. However, T_{b} values reported in Trial 3 were higher than Trial 1 and 2 suggesting that T_{b} hadn't been achieved 24 h post-feeding, or the capture process influences specific dynamic action (SDA) and consequent warming of the viscera.
4. Assuming the T_{rm} is in steady state, heat retention is independent of T_{w} and heat production.

4.5 Trial 4 – The measurement of maximum and basal visceral temperature in commercially cultured southern bluefin tuna (*Thunnus maccoyii*) in response to commercial feeding practices at ambient water temperatures

The results from this Trial demonstrated there was little difference in visceral warming patterns between wild, free roaming SBT that have been implanted with archival tags and SBT used in this study. Specifically, the linear regression results (i.e. slopes of the lines) were very similar to those found in Trial 2 combined scatter plots.

The CSIRO data provided an enhanced view of visceral warming patterns and the relationship with the entire range of water temperatures (T_{w}) experienced in the commercial ranching environment. The data presented in Figure 3.4.1 suggests there is a point where the maximum visceral heating response 'flattens'. As a result, a structural break was investigated in the linear relationship at 21.8^{o}C which was where the gap occurred in data from Trial 2. The structural gap also happened to coincide with the point where maximum visceral warming reached approximately 30^{o}C. At this point there was a

less direct relationship with T_w at higher temperature and a significant difference in the slopes between high and low temperatures, suggesting that this was a level where SBT were managing visceral warming independent of T_w.

The Trial showed that when basal temperature (T_b) is 4 ^oC above ambient T_w, viscera will rise to approximately 6^oC above T_b, white muscle temperature (T_{wm}) will be maintained at 6^oC above ambient $T_{w,}$ and red muscle temperature (T_{rm}) will remain at 30^oC independent of T_w. These results suggest that SBT must manage visceral warming otherwise they would suffer heat stress possibly resulting in cellular damage as seen in rats (Liu et al., 2012). To maintain or manage visceral warming in the ranching environment, SBT would need to either:

- regulate energy intake,
- increase ventilation rate, or
- manage blood flow through the rete mirabile.

Regulating energy intake

As demonstrated in Trials 1 and 2 energy intake influences visceral warming and the duration of visceral warming. Bioenergetic models suggest the rate of heat increment is not always a fixed proportion of gross energy intake and values reported range between 3% and 41% for fish fed natural diets and 11% and 29% for fish fed manufactured diets, and increases with meal size and body weight unless the ration is fixed (Beamish and Trippel, 1990). The SBT ranching industry currently uses feeds comprising baitfish sourced from the local sardine fleet or overseas. There are relatively no acceptance issues, and it is economical whereas manufactured diets have limitations in respect to cost, handling, performance, and acceptability. One of the biggest issues in the development of a commercial manufactured diet is the weaning period for diet acceptance. This weaning period occurs primarily in warm water conditions when SBT are transferred to static ranching pontoons and may last up to five weeks. While previous research has focussed on weaning periods in SBT associated with manufactured diets, underlying issues of health and performance may have not be linked to acceptability but rather to dietary energy and associated visceral warming responses. If consideration is given to the nutritional profile of

feeds, on average, local sardines and manufactured diets have an energy content of 495 kJ and 1470 kJ per 100 g respectively (Ellis and Rough, 2005).

It has been reported that the maximum baitfish consumed by SBT raised in commercial conditions is up to 3 kg day^{-1} whereas the best intakes for the same size SBT fed manufactured diets is up to 1.8 kg day^{-1} (van Barneveld and Ellis, 2007). If energy intake for these feed types are compared this equates to 14850 kJ for baitfish compared with 26460 kJ for manufactured diets. Furthermore, there has always been a lag phase with feeding manufactured diets linked to weaning periods, and coincides with cooling T_w. During periods of cooler water, SBT increase their intake of manufactured diets (Glencross et al., 2002; Gordon et al., 2006) until intakes stabilise with the falling T_w and they reach the same body condition index as SBT fed baitfish.

Anecdotally, feed intake drops in commercial SBT ranching operations when the T_w nears 24^oC suggesting that SBT manage visceral warming by reducing feed intake to ultimately minimise/manage heat production. A similar behaviour is exhibited by Atlantic bluefin tuna (*T. thynnus*) which feed less at spawning grounds than in tropical waters (Carey et al., 1984). Both of these behaviours suggest tuna manage visceral warming through feed intake, and that SBT have the ability to detect dietary energy or vary intake to meet physiological constraints within the ranching environment, and either expend or conserve heat.

Increase ventilation rate

When juvenile tuna acquire or transition to endothermy it is accompanied with the ability to generate and retain heat. This change correlates with a declining surface area to increasing body area and girth (Graham and Dickson, 2001). It has been reported that Pacific tuna (*T. orientalis*) (Kitagawa et al., 2004; Kitagawa et al., 2007), and Bigeye tuna (*T. obesus*) (Holland et al., 1992; Malte et al., 2007) dive below the thermocline to seek cooler waters as a behavioural mechanism to manage visceral warming. Although the mechanism of thermoregulation or heat transfer is not explained (Brill et al., 1994), it would more than likely be associated with heat loss primarily across the increased surface area of the gills with the cooling of the blood to manage visceral warming as opposed to heat transfer through the reduced surface area and insulating capabilities of the skin. However,

according to Brill et al. (1994) tuna are not capable of managing heat in this context as the processes are decoupled in tunas by the presence of vascular counter–current heat exchangers even though all other teleosts are capable of aerobic heat production and loss via the gills.

Mathematical models predict that tunas could overheat during strenuous activity and are possibly activity-limited in warmer water (Brill et al., 1994). It is also believed that large Pacific bluefin tuna (*T. orientalis*) need to inhabit cooler waters to avoid overheating (Kitagawa et al., 2006). In SBT ranching operations where net walls are 10 m in depth and thermoclines do not exist, SBT are unable to manage visceral warming by diving into cooler water. Ranched SBT experience water temperatures between <14^{o}C and >24^{o}C and evidence suggests SBT possibly eliminate excess heat generated through digestion and SDA by increasing heat loss across the gills.

Similar behaviour has been documented in SBT in a mesocosm respirometer whereby high lipid content sardines induced the greatest postprandial metabolic demand and consequent faster post-feeding swimming behaviour to increase ventilation volume to facilitate oxygen uptake (Fitzgibbon et al., 2007; Fitzgibbon et al., 2008). To increase ventilation fish must either swim faster or open their mouths wider and flood their gills to absorb as much oxygen as possible (Parsons and Carlson, 1998; Carlson and Parsons, 2001). It is possible that this behaviour may also be used to manage visceral warming.

Large SBT (~50kg) have been observed to swim slowly around the edges of commercial ranching pontoons with their mouths wide open in the opposite direction to the school during summer months. Given that large SBT have greater heat retention ability (Fitzgibbon et al., 2008), lower gill surface to body size, thicker visceral walls, more insulating subcutaneous fat and increased thermal inertia (Graham and Dickson, 2001), this behaviour is likely to be a response to overheating. By swimming against the direction of the school, they take advantage of the current generated by the school which allows them to conserve energy and reduce any heat generated by SDA as well as minimise further heat production through swimming and reduce body temperature by using their gills as counter current heat exchangers.

Manage blood flow through the rete mirabile

It has been hypothesised that bigeye tuna (*T. obesus*) have extensive physiological thermoregulatory abilities that allow them to change blood flow patterns in the counter-current heat exchangers to manage visceral warming (Holland et al., 1992). Graham and Dickson (2001) suggest that the rete arterial and venule walls have layers of smooth muscles which may provide a mechanism to regulate blood flow. Ultimately, when the viscera warm, tuna must manage and regulate this heat. While extant literature suggests that it is not possible to manage visceral warming due to the decoupling of the process by the presence of vascular counter-current heat exchangers (Brill et al., 1994), tunas dive to cooler waters to thermoregulate (Kitagawa et al., 2004; Kitagawa et al., 2006; Kitagawa et al., 2007). It therefore may be argued that the gills must be used as counter-current heat exchangers to manage excess heat and redirect or minimise blood flow through the rete mirabile or a combination of these processes although no studies have shown circulatory shunts around retia (Graham and Dickson, 2001).

Summary

The Trial demonstrated that

1. There is no significant difference between the relationship of linear models of basal and maximum visceral temperature developed through research and commercial data:
    a. Responses from implanted archival tags used in this study and wild free-roaming SBT are consistent suggesting that the physiology of wild SBT and ranched SBT are the same.
2. There is a maximum water temperature beyond which the linear relationship associated with basal and maximum visceral temperature is compromised:
    a. At this water temperature the maximum visceral heat is 30^{o}C.
3. The structural break introduced to the analysis is an indication of a value at which the relationship with water temperature is very weak:
    a. The water temperature value is approximately 22^{o}C.

4. SBT must manage visceral warming when water temperatures near 22^{o}C to prevent heat stress:
    a. Excess heat generated either through visceral warming or SDA must be managed by:
        i. regulating feed intake,
        ii. using the gills as counter-current heat exchange, and/or
        iii. manipulating blood flow in the rete mirabile.

4.6 Trial 5 - Feed intake, FCR, growth and proximate composition of southern bluefin tuna (*Thunnus maccoyii*) fed four diets during three periods over an 18 week period: *Can feed intake from visceral warming patterns be predicted?*

This discussion is presented in two parts; the first part focuses on SBT performance in respect to feed intake, growth, condition and nutrition assessment; the second part addresses feed intake in relation to visceral warming patterns and baitfish energy content.

SBT performance and nutrition assessment

The impact of tagging on initial feeding is consistent with catch and handling of northern bluefin tuna *(T. thynnus,* L.) (Tičina et al., 2007) and rainbow trout (*O. mykiss*) (Meka, 2004; Meka and Margraf, 2007). Some SBT were found to have negative SGR values in that they lost weight in the first period identical to negative SGR values recorded in northern bluefin tuna (*T. thynnus*), 44 to 53 days post-transfer resulting in a decrease of condition index and consequent weight loss (Tičina et al., 2007).

Condition index (CI) is a length weight relationship as described in Section 2.5. The greater the CI, the greater the energy retained by the fish as lipid reserves. CI is therefore used as a general guide by industry to assess SBT growth performance. A mean CI score above 24 for a harvest, or collectively for a pontoon (growing unit), or across all pontoons is an accepted industry standard. This CI value indicates that SBT growout requirements have been met and the SBT carcass is at a standard that is well received by the Japanese sashimi market.

The results of this Trial indicate that in the first Period there was no significant CI difference between the dietary treatments although the trend suggests that Treatment 1 (T1) and Treatment 2 (T2) performed better than Treatment 3 (T3) and local sardines (T4).

Through Period 2 the results in T1, T2 and T3 suggest that feeding a medium lipid and protein diet appears to produce a CI that meets industry standards whereas T4 did not meet this standard. The difference in CI is likely due to the difference in dietary energy of the feeding regimes.

Period 3 had an interesting effect on condition index. Fish receiving T3 received the LP/HL diet and had lower intakes whilst fish receiving T2 were switched to a HP/LL diet and had a slightly higher intake. The results showed that both T2 and T3 had a lower condition index at the end of Period 3 than at the sampling at the end of Period 2 (albeit the difference was small). This result is consistent with industry experience in that SBT reach condition and as dietary intake drops and so too does condition index. Treatment 1 showed an increasing condition index (albeit a small increase) at the completion of Period 3. A consistent MP/ML was also shown to result in continual improvement in SBT performance. T4 continued to have the highest intake and met the accepted industry condition index of 24 at the completion of Period 3 demonstrating that SBT will achieve a CI that meets an accepted industry standard providing all growth and physiology requirements have been met.

Similar condition indices to those recorded in this Trial have been reported for captured and conditioned Atlantic bluefin tuna (*T. thynnus*). Atlantic bluefin tuna that had been held for five months and fattened on baitfish had a mean condition index of 19.9 and maximum of 27.5 (Aguado-Giménez and García García, 2005). Tičina et al. (2007) reported slightly higher mean and maximum condition indices of 23.3 and 33.1 respectively, for *T. thynnus* that had been held in cages and fattened for 511 days on bait fish. However, the fish were much smaller than in the study by Aguado-Giménez and García García (2005) with an average initial weight of 6.4 kg and final weights ranging from 20 kg to 43 kg as compared to an average final weight of 167 kg in Aguado-Giménez and García García (2005) study.

The trial demonstrated that diet switching is not necessarily the best way to achieve an industry accepted CI standard. Maintaining a constant and balanced supply of protein and lipid appears to be the most desirable feeding strategy for SBT if the requirement is to produce market ready fish quickly. It is important to note that the poorest CI performance

was achieved in T4. This treatment (local sardines) resulted in a lower condition index than all other dietary treatments for the entire experiment.

This Trial indicates that dietary lipid content has an effect on SBT performance and behaviour. Although there was no significant difference between intakes expressed as FI or total intake (kg per SBT per day) in any Period, the data from Period 1 suggests that there is a trend in T2 (HL/LP diet) where high lipid diets have a negative impact on feed intake. The same trend occurs in Period 3 suggesting a lipostatic feedback response.

High-energy feeds, with increased lipid concentrations, are often used in salmon farming for protein sparing and to improve feed to gain ratio. However, fish are thought to regulate ingestion to meet their energy and nutrient intake requirements (Jobling and Johansen, 1999; Johansen et al., 2003). Feeds with excessive lipid content will lead to increased adiposity, and are likely to exert a negative feedback on feed intake via lipostatic regulation mechanisms (Johansen et al., 2002).

An inhibitory effect of elevated body lipid on feed intake in fish has been reported in many studies (Metcalfe and Thorpe, 1992; Jobling and Miglavs, 1993; Shearer et al., 1997; Jobling and Johansen, 1999; Silverstein et al., 1999; Silverstein and Plisetskaya, 2000; Johansen et al., 2002; Johansen et al., 2003). Studies on Atlantic salmon (*S. salar*) have investigated whether feed intake is under lipostatic regulation by producing high lipid and low lipid fish by feeding diets with different lipid concentrations and thereafter observing the intake of the two groups when offered high and low lipid feeds (Johansen et al., 2002; Johansen et al., 2003). Researchers found that salmon had a general preference for leaner feed irrespective of adiposity level, but leaner fish ate more feed, grew faster and deposited more body fat than their fatter counterparts, and over time body compositions converged among treatments, and differences in feed intake abated, indicating lipostatic regulation of feed intake.

According to the model of lipostatic regulation of feed intake, feed consumption of an animal will be negatively correlated to its amount of adipose tissue (Kennedy, 1953; Loftus, 1999; Woods and Seeley, 2000; Young et al., 2005; Geurden et al., 2006; Ruohonen et al., 2007). In contrast, intake increased in European sea bass (*D. labrax)* fed diets high (30%) in lipid (Peres and Oliva-Teles, 2001). In general however, high lipid feeds have been shown to depress performance in European sea bass (*D. labrax)* (Boujard et al., 2004) and Polka-dot

groper (*C. altevelis*) (Williams et al., 2006). Considering the mechanism of adiposity level that regulates feed intake it seems highly unlikely that this was the driver behind reduced feed intake as the condition index ranged between 18 and 19 at the beginning of the trial in T2 and more than 24 in T3 in the last Period.

Anecdotally industry feed intake rates are reduced when high lipid baitfish are used. It is possible that this is associated with limitations of expending visceral heat or alternately dietary requirements are being met or both. This reinforces the regulatory role of dietary energy in food intake, and is particularly relevant for manufactured feeds which are energy dense. Manufactured feeds with high lipid levels greater than 12% may result in reduced intake by SBT.

There is no discernable difference in absolute weight or length increase for all treatments in all Periods which may be a result of the mixed cohorts used in the Trial. The specific growth rate provides a balance to growth performance and demonstrates that T1 displayed the best SGR across all three Periods. The SGR analysis indicates that dietary energy (>7% lipid) provided to T2 and T3 shows a distinct better performance in Period 1 and possibly relates to sufficient energy being supplied to T1 and T2 to meet physiology, growth and retained energy (condition) requirements. The dietary energy provided to T3 and T4 are likely to only have met physiology and growth requirements and partially met retained energy requirements.

In Period 2, results suggest that compensatory growth occurred in T3 and T4 where both these treatments showed increasing growth against falling water temperature. These results contrast with the values attained by T1, T2 and Period 3 in that growth was correlated with falling water temperature as also demonstrated by Glencross et al. (2002).

If an assumption is made that the MP/ML diet is ideal for SBT based on these Trial results, then diet switching in Period 2 resulted in feeding regimes that met growth and condition requirements. Fish receiving these feeding regimes went through a compensatory growth phase as evident by the high SGR and low FCR for this Period. In respect to T4, SBT gradually gained condition once growth requirements had been met.

Compensatory growth in salmonids has been well researched (Jobling and Koskela, 1996; Bull and Metcalfe, 1997; Jobling and Johansen, 1999; Nikki et al., 2004). It is generally

accepted that fat reserves are gradually restored during catch-up growth and once the required threshold is reached compensatory growth ceases (Johansen et al., 2001).

Carter et al. (1998) provided SGR for SBT which were much lower and demonstrate the considerable advances in research that have occurred. The specific growth rates recorded in this research (Figure 3.5.6) for two to three year old SBT after 126 days were 58% to 63% greater compared to Atlantic bluefin tuna (*T. thynnus*) held for 540 days (Katavic et al., 2003), and 62% to 67% greater compared with two year old *T. thynnus* held for over 500 days that were tagged, whereas the impact from tagging was reduced (49% to 55%) with non tagged fish. Furthermore, *T. thynnus* of the same age group as those used in this thesis and held for a similar Period had specific growth rates of 0.31%/day in summer and 0.20%/day in winter (Tičina et al., 2007). This suggests growth rates in this study were better than in *T. thynnus* but tagging has a direct impact on SGR (Tičina et al., 2007). For this reason the impact of tagging needs to be considered in context of the results of this Trial.

There was a period of this Trial between 30 May 2005 to 13 June 2005 where intakes were suppressed (Figure 3.5.13). While this may be attributed to diet switching, the impact was discernable across all treatment groups and is more likely due to blood fluke (*Cardicola forsteri)* infection (Hayward et al., 2010; Cribb et al., 2011; Dennis et al., 2011). Following the infection, feed intakes increased against falling water temperatures, possibly as a compensatory growth effect to make up for lost growth across all treatments.

At the beginning of the third treatment Period feed intakes stabilised and reflected the falling water temperature consistent with previous research (Glencross et al., 2002). In the first treatment Period there were changes in the lipid concentrations in SBT flesh across all treatments. Specifically, T2 received the high lipid low protein diet and showed a significant increase in lipid content compared with other treatments. The increase in flesh fattiness may have been the result of over consumption of lipid to gain limiting protein as demonstrated in European whitefish (*C. lavaretus*) (Ruohonen et al., 2007).

Alternatively, it may have been a result of storing lipid reserves consistent with the objectives of commercial ranching, but it is more likely to be a direct result of SBT physiology (Caton, 1991; Gunn et al., 2002).

T2 displayed lower moisture content during Period 1 suggesting moisture is displaced by lipid. There were no other differences in lipid within Periods but between Periods there was a gradual increase in flesh lipid content which reflects the changes in CI with protein being held constant across all Periods. One exception was a lower protein value for T3 in Period 1 which may be related to protein being used as an energy source. However this seems unlikely due to the response of fish in T4 that received similar dietary energy and lipid.

The reported wet weight food conversion ratios for this Trial (Figure 3.5.12) are significantly higher than reported in other SBT studies (6.3 – 9.3:1) (Gunn et al., 2002) and are inconsistent with values reported by industry (9 – 11:1). Reasons for the high FCR include pre-condition, start time of the trial, handling, and possible over feeding.

Food conversion ratios (FCR) displayed in Period 1 showed two interesting trends. Treatment 2 showed the lowest FCR and coupled with the same SGR as T1, consistent absolute growth and equal CI to that of T1, results suggest that medium to high lipid feed in the first Period will achieve the best outcome. The second trend is that low lipid diets will result in increasing FCRs with retained energy being compromised but not growth. In the second Period the FCR for all treatments except T1 dropped suggesting compensatory growth was occurring resulting in better SBT performance. The final Period showed increasing FCRs for all treatments except T1 suggesting that dietary changes solicit changes in FCR and sufficient energy still needs to be provided to meet physiology, growth and retained energy requirements as shown in T4.

Medium to high lipid diets comprise mainly expensive imported high lipid feed. The relative cost of local sardines may still make them the most cost-effective feed source compared with imported baitfish providing SBT ranching companies wish to hold SBT for 18 weeks and meet the acceptable CI standard. Trial results indicate that three out of the four treatments reached the accepted industry CI standard after 12 weeks and companies should assess whether holding SBT for 18 weeks is cost-efficient and justifies the risks of holding fish longer such as mortalities, increasing FCR and reducing CI.

Application of costs to the relative treatments used in this experiment and the use of *FORMU-BAIT*© will allow companies to make informed decisions about the price sensitivity of all baitfish to meet feed specifications.

Visceral warming and assessing intake

The purpose of this component of Trial 5 was to assess SBT performance linked to nutrition through implanted archival tags. As research and commercial constraints made it impossible to undertake this, the objective of the trial was amended to assess visceral warming patterns and determine whether feed intake could be predicted. Results indicate that there is very little difference between treatment groups and Feed Measure (FM) with the exception of T3, reinforcing the view that visceral heat is regulated. It is possible that the mean result for T3 is either due to tag placement or feeding patterns so that this result has been considered with caution.

The trend in data shows that as water temperature drops, feed intake decreases and visceral warming increases. This suggests that visceral warming (expressed as FM) is not necessarily a direct function of feed intake but is regulated independently of feed intake and likely serves as a function of heat conservation. This result also supports the theory that SBT expend visceral heat when the water is warm and conserve heat when the water is cool and that visceral heat is largely independent of intake.

The models developed in this thesis to predict feed intake produced erratic results and were not considered as a true reflection of the mean feed intake for each treatment and were not pursued further. Reasons for the erratic responses are likely to be: feeding behaviours as identified in Trial 2; feed consumption at a pontoon level being greater than what was modelled; absence of interaction between physiology requirements and dietary requirements. The application of archival tags to measure feed intake requires further research and modelling before accurate models may be developed.

Summary

The trial demonstrated that:

1. Maintaining a consistent feed nutritional profile delivers the best results in respect to SBT performance (growth, condition index and FCR).
2. More than 7% lipid is required in the diet to optimise specific growth rate in the first six weeks.

3. Feeding diets comprising greater than 10.5% lipid maintains optimum specific growth rates but reduces feed intake. This is influenced by:
    a. High lipid/energy content of the diet and a feedback mechanism in the SBT, or
    b. Dietary energy influencing visceral warming and the fish regulating intake to regulate visceral warming.
4. Feeding high lipid diets in the early phase of ranching SBT may lead to increased flesh lipid levels.
5. Specific growth rates in SBT are significantly better than Atlantic bluefin tuna of the same age and size regardless of treatment.
    a. Tagging and associated stress of handling impact research results.
6. Compensatory growth occurs and is likely to be related to:
    a. SBT condition
    b. Tagging and handling
    c. Blood fluke infection
    d. Change in diet
7. Relative costs for commercial feeding strategies can be assessed based on proposed harvest strategies.
8. Intake cannot be predicted based on visceral warming patterns, however:
    a. Dietary energy does not influence visceral warming expressed as Feed Indicator.
    b. Falling water temperature is associated with decreased feed intake and increased visceral warming.
    c. There is a point in visceral warming where energy thresholds are reached.

4.7 Trial 6 - – Measurement of visceral warming patterns in commercially grown southern bluefin tuna (*Thunnus maccoyii*) in response to two feeding regimes

Sekol Farmed Tuna fed a combination of imported frozen high lipid feeds with local sardines twice a day whilst *Kistuna* fed mainly fresh locally caught sardines up to six times per day. Both companies fed on average six days per week.

Unfortunately data provided by *Sekol Farmed Tuna* did not align with the data provided by tags in respect to visceral warming patterns. One of the issues faced is data integrity when aligning data that is collected at two different time points especially when archival tags are downloaded at the completion of the trial. According to the archival tags retrieved from *Sekol Farmed Tuna,* SBT ate 20.7% more than what they were offered. Whilst there was an occasion where wild baitfish were known to have entered the pontoon and were consumed near midnight it would appear that extra feeding events were associated with recording errors as opposed to the fish regularly feeding on wild baitfish.

Feed Indicator response to feeding regime (Company)

The analysis showed there was no difference between companies and feed measure (FM) response, with the exception that both companies had a significantly lower FM response during Period 2 of the analysis. This lower feed measure response is more than likely associated with blood fluke (*C. forsteri)* infection (Hayward et al., 2010; Cribb et al., 2011; Dennis et al., 2011). The slight difference in FM may be associated with the *Kistuna* fish being fed on low energy local sardines in Period 1 or the regular addition of cold baitfish resulting in the lowering of visceral temperature.

Feed events, feed consumed and FM (visceral warming response)

There was no difference in FM in response to company feeding strategies (Figure 3.6.4), however the data suggest that for *Kistuna* to achieve the same FM response as *Sekol Farmed Tuna* the company must feed SBT either five or six times per day.

This Trial has for the first time shown a detectable change in FM according to feeding regime. It could be assumed the high energy profile of baitfish delivered by *Sekol Farmed Tuna* was sufficient to meet physiology requirements based on two feeds per day whereas the low energy local baitfish fed by *Kistuna* needed to be delivered to SBT five times per day

to meet the same physiology requirements. However, the regular addition of cool food to the stomach may have resulted in a cooling effect of the viscera as reported by Gunn et al. (2002). Regardless of whether the nutritional content of the feed was sufficient to provide growth and condition or the temperature of the food, the data has highlighted an interaction between feed frequency and visceral warming.

It is recognised that the timing and delivery of an optimal diet are important and that feeding to satiation with an optimised diet produces better growth rates than sub-satiation feeding (Watanabe et al., 2000). Significant research has been undertaken in other aquaculture species to identify the ideal feeding frequency. In one study rainbow trout (*Oncorhynchus mykiss*) were fed to satiation two, three, four and six times per day for eight weeks. The results showed that weight gain, growth rate and feed intake and final body weight decreased with decreasing frequency of feeding events; it was recommended that fish be fed six times per day (Tuerker and Yildirim, 2011). Olive flounder (*P. olivaceus*) fed one, two or three meals daily and one meal every two days to satiation for seven weeks showed that three feeds per day produced the best results in respect to maximal growth performance (Lee and Pham, 2010). Further research revealed that when fed one, two, three or four times daily weight increased according to feeding frequency but there were no differences observed between fish that were fed three and four times per day (Lee et al., 1998). In another study Pikeperch (*Sander lucioperca*) were fed either one, three or six times per day to satiation with the best results achieved at three times per day, as protein and lipid retention was found to be lower than when fed six times per day (Wang et al., 2009).

Juvenile hybrid striped bass (*Morone saxatilis*) were fed one, two meals daily, two meals in the day and one at night, three times per day, and four times per day. The increased feed frequency led to increased FCRs and the group that was fed three times per day with a six hour interval in between had the best SGR (Liu and Liao, 1999).

A study by Andrews and Page (1975) assessed the effects of feeding frequency on channel catfish (*Ictalurus punctatus)* based on one, two or four times per day to satiation and found that growth was reduced in groups fed one time per day and were not enhanced by feeding four times per day.

This Trial suggests that food intake and not food utilisation was the limiting factor for visceral warming. The literature shows that while every species is different in its feed requirements, some aquacultured fish can be fed too much. This study has shown that SBT are different to other fish due to their physiology and it is difficult to draw any conclusions from previous studies other than tuna.

Results of Trial 1 show that dietary energy affects the time taken for the viscera to reach t_{max}, when gastric evacuation occurs (Carey et al., 1984; Gunn et al., 2002). The relationship with dietary energy and FM will reach an end point and plateau and no further FM increase can be registered. Furthermore, dietary energy influences the duration of visceral warming: the greater the energy, the longer the duration of visceral warming. Putting this information into context, it may be assumed the low energy diets provided by *Kistuna* were being digested faster than the high energy diets offered to *Sekol Farmed Tuna*. This resulted in SBT requiring more feeds to meet the same level of FM and suggests, in part, that to meet the upper threshold of FM these fish need to be fed five times compared with twice for the *Sekol Farmed Tuna*. Alternatively, it is possible that due to the SBT being fed five to six times per day, *Kistuna* SBT feed intake volume reflects the same amount of feed consumed by *Sekol Farmed Tuna* fed twice per day resulting in a difference in FM. Given the results from Trial 5 this seems unlikely as the results would suggest links between dietary energy and intake.

Feeding can influence circadian rhythms in fish (Spieler, 1992; Sanchez-Vazquez et al., 1997) and the timing of feed delivery may influence the time and intensity of endocrine cycles involved in the physiological regulation of feeding which may affect processes involved in energy and nutrient use and storage(Bolliet et al., 2001). It was also the view of the *Sekol Farmed Tuna* farm manager that the diet delivered in the afternoon should be high in lipid as it gave the SBT a longer time to digest the meal. He also believes that SBT empty their 'bowels' every morning when he arrived at the pontoon to feed them and suggested that it was a feeding behavioural response to make ensure that they 'make the most out of every morning feeding opportunity' (D. Warland 2009, pers comm., September 18).

This observation is consistent with observations of wild SBT that will feed until satiated in the wild and make the most out of every feeding event and then process the feed which is consistent with current views on fish feeding behaviour (Madrid et al., 2001).

The general feeding pattern showed that when SBT ate meals that were not on offer the FM was at its highest. When meals were skipped FM was lower. The results are likely to be more reflective of the data rather than absolute as in general SBT would have either eaten what was offered to them or ate less. The interesting aspect of this analysis is the FM difference between the companies: *Sekol Farmed Tuna* had a consistently higher FM for two feeds compared to *Kistuna*. As SBT eat less the FM decreases.

Summary

This Trial provided some insights into visceral warming in commercial grown SBT subjected to different feeding regimes. The results show that:

1. There is no difference between feeding regime and FM.
2. There is a difference between feeding events and FM and is likely to be associated with diet energy.
3. Missed meals will result in lower FM.

Chapter 5 - Conclusion

The aims of this thesis was to measure SBT visceral temperature patterns and explore relationships between visceral heat change, nutrient supply, feed frequency and efficiency. This was accomplished in six trials with different but related objectives building on existing research in this field and exploring assumptions within previous nutrition studies.

Trial 1 determined that archival tags implanted into the visceral cavity of two to three year old free swimming SBT could record visceral warming patterns. Dietary energy is the main driver of visceral warming patterns and when SBT were fed a single meal up to 1kg very good linear regression relationships existed between dietary energy and visceral warming, the time taken to digest a meal and the time taken to reach peak visceral temperature. Where SBT consumed more than 1 kg of baitfish or the energy value exceeded 7000 kJ the measure of visceral warming would plateau suggesting a heat control mechanism. Although the time for visceral temperature to peak was influenced by dietary energy, size of baitfish may have influenced this result and further investigations are required. However, when SBT consumed a meal feed intake could be predicted providing dietary energy was known. The higher the dietary energy the more certainty existed in predicting feed intake.

As Trial 1 was performed at cool temperatures, the direction of Trial 2 was undertaken in contrasting water temperature profiles using consistent dietary energy and assessed visceral warming in response to one, two or three daily feeds. This was to develop models that could be applied in the research to assess feed intake in manipulated dietary energy feed Trials and commercial ranching pontoons.

In Trial 2 dietary energy remained the main driver influencing relationships with visceral warming and water temperature. Confidence in results allowed feed frequency data to be pooled and assessed independently for both treatment Periods. This resulted in two different general liner models with a stronger relationship in the Period of cool water. Visceral warming was greater during cool water when feed intake was low, yet visceral warming was lower with increased feed intake in warm water, therefore demonstrating visceral heat was being expended during warm water and conserved during cool water and emphasised that SBT physiologically manage visceral heat. Furthermore, time taken to

reach maximum visceral temperature was not a reliable indicator to predict feed intake due to more feed being added to the stomach before it had time to clear and different quantities of food consumed at feeding events. The measurement of visceral warming patterns from Trials 1 and 2 suggest feed intake could be predicted in cool water temperatures with measurable certainty providing dietary energy was known.

Data obtained from basal and maximum visceral temperatures measures in Trial 1 and Trial 2 were assessed to examine visceral warming patterns in relation to ambient water temperatures. The relationship of basal and maximum visceral temperature with ambient water temperature was linear when <20^{o}C and could be predicted with certainty, but above this temperature the relationship was uncertain. The data showed a level where maximum visceral temperature flattened (approximately 30^{o}C) and this relationship was explored further in Trial 4.

However, the results of Trials 1 and 2 suggested there might be additional factors governing basal and maximum visceral warming. Trial 3 investigated the temperature profile of red and white muscle and viscera in response to three feeding regimes and ambient water temperature. Red muscle was maintained at approximately 30^{o}C independent of ambient water temperature, white muscle was maintained at approximately 6^{o}C above ambient water temperature and basal visceral temperature could be predicted with confidence in relation to ambient water temperature providing the viscera had sufficient time to return to basal post feeding (> 24 h). These results suggest SBT physiologically thermoregulate body tissues in response to ambient water temperature.

Trial 4 assessed the response of commercial feeding practices on SBT health and physiology by using archival tags implanted into the visceral cavity of two to three year old free swimming SBT in response to ambient water temperature. Basal visceral temperature was maintained at 4^{o}C above ambient water temperature and maximum visceral temperature was recorded up to 10^{o}C above ambient water temperature. Both aspects of visceral warming patterns maintained very strong linear relationships with ambient water temperature up to approximately 20^{o}C. In water temperatures above 20^{o}C the linear visceral warming relationships was weak. Combining research outcomes from Trials 3 and 4 reveals if the water temperature is 20^{o}C, basal visceral temperature is therefore 24^{o}C, white muscle temperature is 26^{o}C and red muscle is maintained at 30^{o}C suggesting SBT would

have a requirement to expend excess visceral heat as a consequence of food digestion and would likely rely on gills being used as counter current heat exchangers and redirecting blood flow from the rete mirabile. This demonstrates SBT physiologically regulate body temperature. Furthermore, there were no differences in visceral warming patterns from SBT that had been tagged in the wild and SBT that had been tagged on arrival in the farming zone.

The objectives of Trial 5 were to utilise *FORMU-BAIT*© feed optimisation software developed by an Aquafin-CRC project to formulate combinations of baitfish diets varying in protein and fat content to optimise SBT growth and feed intake over the farming season. Trial 5 considered four feeding strategies including diet switching reflecting current industry feeding practices. Maintaining a consistent feed profile (> 7% lipid) optimises SBT performance in respect to growth, intake, condition and FCR. There did appear to be an advantage in feeding medium to high lipid feeds early in the Trial if meeting an early marketing opportunity. Feeding high lipid diets at the end of the Trial reduces feed intake and results in decreasing SBT condition index (albeit a small difference). Specific growth rate showed SBT performing better than Atlantic bluefin tuna and previous SBT research results, demonstrating research and industry have made significant nutrition advances in improving SBT performance. Compensatory growth occurred during the Trial and was a likely response due to SBT condition, tagging and handling, blood fluke infections and diet switching.

The general linear model developed in Trial 2 was applied to visceral warming patterns in Trial 5 to determine feed intake based on known dietary energy. The main constraint to develop a predictive feed intake model is when a certain dietary energy threshold has been met in relation to water temperature there is no more increase in visceral warming to reflect dietary energy intake. Furthermore in Period 1 of Trial 5, mean feed intakes were high and visceral warming was low whereas in the last trimester Period mean feed intakes were low and visceral warming was higher, further emphasising a physiology response to manage visceral heat.

Therefore, predicted feed intake was inconclusive and did not reflect mean pontoon/treatment feed intake results.

There are many complicating factors preventing archival tags from predicting intake with measurable certainty as visceral heat is managed to meet SBT physiology requirements. Visceral warming is influenced by dietary energy, temperature of the feed, feed frequency, feeding behaviour, and ambient water temperature. Whilst it was not tested in this thesis the age of fish and condition index of SBT may have an influence.

The objective of Trial 6 was to assess visceral warming patterns in response to feeding events when SBT were fed two or up to six times per day. There was no difference in visceral warming patterns when SBT were fed either two or six times per day. However visceral warming differences were noted when SBT that were accustomed to being fed six times per day were not fed five or six times per day. The difference may be related to dietary energy as the company that fed twice per day typically fed high lipid imported feed blended with local sardines whereas the company that fed up to 6 times per day fed mainly low lipid local sardines. Understandably missed meals resulted in lower visceral warming values.

Industry implications

Archival tags for measuring feed intake

There is no commercial benefit at this time to use archival tags inserted into SBT to measure feed or energy intake. Despite previous research suggesting that this could be advantageous, archival tags are limited in their application for refining feeding strategies.

Avoid handling and tagging

It was demonstrated that handling and tagging SBT has an adverse effect on SBT performance in respect to growth, intake, condition and FCR in the short term and should be avoided.

Whilst physiology measurements tended not to be compromised, growth performance in Trial 5 and experience with handling tuna around the world suggests tagging should be avoided. Thus all tagging studies should be undertaken with as much care as possible and results treated with caution when demonstrating SBT growth and performance.

Upper temperature limit

Red muscle temperature was recorded at approximately 30^{o}C and is independent of water temperature, white muscle was recorded at 6^{o}C above ambient water temperature and the basal visceral temperature was 2^{o}C above ambient water temperature during warm water temperatures and increased to 4^{o}C during cooler water temperatures.

There is an upper water temperature threshold in the range of 20 – 22^{o}C where visceral warming patterns change resulting in possible heat stress. In the wild, SBT have the option to swim below the thermocline to expend excess heat, however this option is removed in commercial SBT ranching operations. During periods of water temperatures >20^{o}C operators should manage SBT dietary energy intake to reduce the impact of SBT managing visceral heat. The size of SBT also needs to be considered as the smaller the SBT the less insulation and increased ability to expend heat through the gills.

Managing blood fluke infections

The mortality event currently experienced by industry six to eight weeks post transfer is possibly associated with blood flukes *C. forsteri* and *Cardicola orientalis*. These parasites have an intermediate host that lives in the sediments, the primary host is SBT and it is believed blood fluke cercaria enters fish through the skin (Cribb et al., 2011). The infection impacts on the cardio-vascular system resulting in mortalities or reduced and impaired feeding.

A concern for SBT farm mangers is the relationship between swimming speed as reported in Fitzgibbon et al. (2008) in response to high lipid diets. If SBT require more water to flow across the gills for oxygen and to expend visceral heat possibly increase the potential for blood fluke infection.

At water temperatures >20^{o}C operators should consider feeding low lipid diets and reduce feed frequency.

Compensatory growth

The feeding Trial demonstrated that depending on the intended marketing period there is no benefit in feeding high lipid diets in the beginning of the season and this research

suggests SBT will reduce intake and it may be a health hazard. If SBT are fed maintenance diets during periods of warm water, Trial outcomes demonstrate SBT will experience compensatory growth.

Nutritional requirements

Research showed SBT growth performance can be optimised by feeding a constant medium protein and medium lipid diet for the duration of the season until ready for harvest. However, optimising SBT growth performance needs to be considered in context with logistics of feed delivery, sourcing and cost of baitfish, and harvest schedule. The computer program *FORMUBAIT©* can assist with developing feeding profiles to match individual company feeding requirements.

Cost effective feeding and harvest strategies

Performance measures identified in this thesis can inform industry on ideal harvesting schedules. If destined for the frozen market, gains can be assessed in context with economical return, potential risk and exposure.

Development of manufactured feeds

Visceral warming has direct implications on development of energy dense manufactured feeds. For many years research focussed on weaning believing that to increase diet acceptance it required trickery, starvation, and time, when it could be as simple as manufactured feeds being too energy dense for the SBT digestive system.

On a weight for weight basis, manufactured diets contain three times more kilojoules than baitfish therefore trying to get the same feed intakes comparable to baitfish would be out of reach due to SBT physiological constraints. Further work is required in this area to match dietary energy to growing period.

Feeding frequency

Dietary energy and feeding frequency is linked and needs further exploration. The implications from outcomes presented in this thesis suggest the amount of feeds to be given to SBT is dependent on dietary energy.

Future Research

This thesis has revealed aspects of SBT physiology and nutritional requirements. However, there are aspects requiring further research to understand SBT physiology and optimise production. The following priorities are recommended for further research:

Development of a bio-physiology-energetic model

Feed intake prediction models interpreting visceral warming patterns as a guide is not effective. Over the past 15 years there has been a plethora of gathered data that should be synthesised in a desk top study to determine SBT energy/nutritional requirements.

Assessing gut evacuation time

Carey et al. (1984) suggested time taken to reach maximum visceral temperature is linked with gut evacuation through the contraction of stomach muscles. This requires further investigation as it was demonstrated in this thesis that the time taken to reach maximum visceral warming changed with dietary energy.

Baitfish size and impact on visceral warming

The information presented in this thesis suggests dietary energy influences visceral warming patterns. However, the influence of baitfish size on visceral warming patterns requires further investigation as it could lead to significant advances in SBT growth performance.

Investigate cold activated enzymes

A hypothesis put forward by Gunn et al. (2002) suggests tuna digestion is constant regardless of water temperature and the increase in visceral warming in cold water temperatures is attributed to cold activated enzymes in the caecum. Further research is required to determine whether these cold activated enzymes do exist.

Feeding frequency

This research demonstrated digestion is related to dietary energy. However, further research needs to consider SBT growth performance in context with dietary energy and feeding frequency.

Thermal imaging

Measuring red and white muscle and visceral temperature in a resting state should be investigated further. As blood fluke infects and impacts the cardio-vascular system it would seem that this is a very worthwhile research direction.

Summary

This thesis

1. Measured SBT visceral warming patterns in response to baitfish of varying size and energy content to water temperature.
2. Measured SBT visceral warming patterns in response to feed frequency and water temperature.
3. Investigated relationships between red and white muscle, visceral warming and ambient water temperature.
4. Determined basal and maximum visceral temperature in response to ambient water temperature.
5. Determined the impact of different feeding strategies on SBT growth performance.
6. Investigated models that could be applied to measure feed intake.
7. Assessed two industry feeding frequencies to determine efficiency.
8. Informed industry of potential issues that may affect feeding strategies in response to measuring visceral warming patterns.
9. Identified further research based on outcomes.

References

Aguado-Gimenéz, F. and García-García, B. (2005). Changes in some morphometric relationships in Atlantic bluefin tuna (*Thunnus thynnus thynnus* Linnaeus, 1758) as a result of fattening process. *Aquaculture*. **249**, 303-309.

Aguado-Giménez, F., García-García, B., Hernández-Lorente, D. M. and Cerezo-Valverde, J. (2006). Gross metabolic waste output estimates using a nutritional approach in Atlantic bluefin tuna (*Thunnus thynnus)* under intensive fattening conditions in western Mediterranean Sea. *Aquacultural Research.* **37**, 1254–1258.

Altringham, J. D. and Block, B. A. (1997). Why do tuna maintain elevated slow muscle temperatures? Power output of muscle isolated from endothermic and ectothermic fish. *Journal of Experimental Biology.* **200**, 2617-2627.

Altringham, J. D. and Shadwick, R. E. (2001). Swimming and muscle function. In: B. A. Block and E. D. Stevens (Eds.), Tuna physiology, ecology, and evolution. Academic Press, San Diego, pp. 313-344.

Andrews, J. W. and Page, J. W. (1975). The effects of frequency of feeding on culture of catfish. *Transactions of the American Fisheries Society*. **104**, 317-321.

Ang, K. P. and Petrell, R. J. (1997). Control of feed dispensation in seacages using underwater video monitoring: Effects on growth and food conversion. *Aquacultural Engineering.* **16**, 45-62.

Anon. (1993). Report on the joint feasibility study project for southern bluefin tuna farming in Boston Bay, Port Lincoln, South Australia. Government of South Australia, Tuna Boat Owners Association of Australia, Overseas Fishery Cooperation Foundation. 81 pages.

Anon. (2012). Origins of the convention, viewed 20 March, 2012. http://www.ccsbt.org/site/origins_of_the_convention.php.

Attenborough, D. (1979). *Life on Earth*. Boston, MA: Little, Brown and Company. 319 pp.

Azaza, Dhraief, M. N., Kraiem, M. M. and Baras, E. (2010). Influences of food particle size on growth, size heterogeneity, food intake and gastric evacuation in Nile tilapia, *Oreochromis niloticus,* L., 1758. *Aquaculture.* **309**, 193-202.

Bailey, J., Alanara, A. and Crampton, V. (2003). Do delivery rate and pellet size affect growth rate in Atlantic salmon (*Salmo salar* L.) raised under semi-commercial farming conditions? *Aquaculture.* **224**, 79–88.

Bascinarl, N., Cakmak, E., Cavdar, Y. and Aksungur, N. (2007). The effect of feeding frequency on growth performance and feed conversion rate of Black Sea trout (*Salmo trutta labrax* Pallas, 1811). *Turkish Journal of Fisheries and Aquatic Sciences.* **7.1**, 13-17.

Beamish, F. W. H. (1974). Apparent specific dynamic action of largemouth bass, *Micropterus salmoides*. *Journal of Fisheries Research Board of Canada.* **31**, 1763-1769.

Beamish, F. W. H. and Trippel, E. A. (1990). Heat Increment: A static or dynamic dimension in bioenergetic models. *Transactions of the American Fisheries Society.* **119**, 649-661.

Begout Anras, M-L., Beauchaud M., Juell, J-E, Coves, D. and Lagardere, J-P. (2001). Environmental factors and feed intake: Rearing systems. In: D. Houlihan, T. Boujard and M. Jobling (Eds.), Food intake in fish. Blackwell Science, Iowa, USA, pp. 157-189.

Bestley, S., Patterson, T. A., Hindell, M. A. and Gunn, J. S. (2008). Feeding ecology of wild migratory tunas revealed by archival tag records of visceral warming. *Journal of Animal Ecology.* **77(6)**, 1223-1233.

Bestley, S., Patterson, T. A., Hindell, M. A. and Gunn, J. S. (2010). Predicting feeding success in a migratory predator: integrating telemetry, environment and modelling techniques. *Ecology.* **91(8)**, 2373-2384.

Block, B. A. and Carey, F. G. (1985). Warm brain and eye temperatures in sharks. *Journal of Comparative Physiology.* **156B**, 229-236.

Block, B. A. and Finnerty, J. R. (1994). Endothermy in fishes: a phylogenetic analysis of constraints, predispositions and selection pressures. *Environmental Biology of Fishes.* **40**, 283-302.

Block, B. A., Booth, D. and Carey, F. G. (1992). Depth and temperature of the blue marlin, *Makaira nigricans*, observed by acoustic telemetry. *Marine Biology.* **114**, 175-183.

Block, B. A., Finnerty, J. R., Stewart, A. F. R. and Kidd, J. (1993). Evolution of endothermy in fish: mapping physiological traits on a molecular phylogeny. *Science.* **260**, 210-214.

Block, B. A., Keen, J.E., Castillo, B., Dewar, H., Freund, E. V., Marcinek, D. J., Brill, R. W. and Farwell, C. (1997). Environmental preferences of yellowfin tuna (*Thunnus albacares*) at the northern extent of its range. *Marine Biology.* **130**, 119-132.

Block, B. A., Dewar, H., Blackwell, S. B., Williams, T. D., Prince, E. D., Farwell, C. J., Boustany, A., Teo, S. L. H., Seitz, A., Walli, A., Fudge, D. (2001). Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. *Science.* **293**, 1310-1314.

Blyth, P. J., Kadri, S., Valdimarsson, S. K., Mitchell, D. F. and Purser, G. J. (1999). Diurnal and seasonal variation in feeding patterns of Atlantic salmon *Salmo salar* L., in sea cages. *Aquaculture Research.* **30 (7)**, 539-544.

Bolliet, V., Azzaydi, M. and Boujard, T. (2001). Effects of feeding time on feed intake and growth. In: D. Houlihan, T. Boujard and M. Jobling (Eds.), Food intake in fish. Blackwell Science, Iowa, USA, pp. 233-249.

Boujard, T and Leatherland, J. F. (1992). Demand-feeding behaviour and diel pattern of feeding activity in *Oncorhynchus mykiss* held under different photoperiod regimes. *Journal of Fish Biology*. **40**, 535-544.

Boujard, T., Gélineau, A., Covés, D., Corraze, G., Dutto, G., Gasset, E. and Kaushik, S. (2004). Regulation of feed intake, growth, nutrient and energy utilisation in European sea bass (*Dicentrarchus labrax*) fed high fat diets. *Aquaculture.* **231**, 529-545.

Brännäs, E., Berglund, U. and Eriksson, L-O. (2005). Time learning and anticipatory activity in groups of Arctic charr. *Ethology.* **111**, 681-692.

Brill, R. W. (1996). Selective advantages conferred by the high performance physiology of tunas, billfish, and dolphin fish. *Comparative Biochemistry and Physiology*. **113A**, 3-15.

Brill, R. W., Dewar, H. and Graham, J. B. (1994). Basic concepts relevant to heat transfer in fishes, and their use in measuring the physiological thermoregulatory abilities of tunas. *Environmental Biology of Fishes.* **40**, 109-124.

Brown, C. R. and Cameron, J. N. (1991a). The induction of specific dynamic action in channel catfish by infusion of essential amino acids. *Physiological Zoology.* **64**, 276-297.

Brown, C. R. and Cameron, J. N. (1991b). The relationship between specific dynamic action (SDA) and protein synthesis rates in the channel catfish. *Physiological Zoology.* **64**, 298-309.

Bull, C. D. and Metcalfe, N. B. (1997). Regulation of hyperphagia in response to varying energy deficits in overwintering juvenile Atlantic salmon. *Journal of Fish Biology.* **50**, 498-510.

Bushnell, P. G. and Jones, D. R. (1994). Cardiovascular and respiratory physiology of tuna: adaptations for support of exceptionally high metabolic rates. *Environmental Biology of Fishes.* **40**, 303-318.

Calder, M. (1977). Australian South Coast – AUS 00134 and Port Lincoln and Approaches – AUS 00134. RAN Hydrographic Service, Woolongong, NSW.

Campbell, D. (2001). Change in fleet capacity and ownership of harvesting rights in the Australian southern bluefin tuna fishery. In: R. Shotten (Ed.) Case studies on the effects of transferable fishing rights on fleet capacity and concentration of quota ownership. FAO Fisheries Technical Paper 412, pp 134 – 150.

Carey, F. G. (1973). Fishes with warm bodies. *Scientific American.* **228**, 36-44.

Carey, F. G. and Robinson, B. H. (1981). Daily patterns in the activities of swordfish, *Xiphias gladius*, observed by acoustic telemetry. *Fishery Bulletin.* **79**, 277-294.

Carey, F. G., Teal, J. M. and Stevens, E. D. (1984). Bluefin tuna warm their viscera during digestion. *Journal of Experimental Biology.* **109**, 1-20.

Carlson, J. K. and Parsons, G. R. (2001). The effects of hypoxia on three sympatric shark species: physiological and behavioural responses. *Environmental Biology of Fishes.* **61**, 427-433.

Carter, C. G. and Brafield, A. E. (1992). The relationship between specific dynamic action and growth in grass carp, *Ctenopharyngodon idella* (Val.). *Journal of Fish Biology*. **40**, 895-907.

Carter, C. G. and Houlihan, D. F. (2001). Protein synthesis. In: P.A. Wright & P.M. Anderson (Eds), Fish Physiology Volume 19, Nitrogen Excretion. Academic Press, New York. pp 31-75.

Carter, C. G., Seeto, G. S., Smart, A., Clarke, S. and van Barneveld, R. J. (1998). Correlates of growth in farmed juvenile southern bluefin tuna *Thunnus maccoyii* (Castelnau). *Aquaculture.* **161**, 107-119.

Carter, C. G., Bransden, M. P., van Barneveld, R. J. and Clarke, S. M. (1999). Alternate methods for nutrition research on the southern bluefin tuna, *Thunnus maccoyii*: Invitro digestibility. *Aquaculture.* **179**, 57–70.

Carter, C. G., Lewis, T. E. and Nichols, P. D. (2003). Comparison of cholestane and yttrium oxide as digestibility markers for lipid components in Atlantic salmon (*Salmo salar* L.) diets. *Aquaculture* **225**, 341-351.

Castelnau, F. de (1872). Contribution to the ichthyology of Australia. *Proc Zool. Acclim. Soc. Vict.* **1**, 29–248.

Caton, A. E. (1991). Review of aspects of southern bluefin tuna biology, population and fisheries. In: R.B. Deriso and W.H. Bayliff (Eds), World meeting on stock assessment of bluefin tunas: strength and weaknesses. Inter-American Tropical Tuna Commission, Special Report No.7 La Jolla California, pp 357.

Chen, W.-M. and Tabata, M. (2002). Individual rainbow trout can learn and anticipate multiple daily feeding times. *Journal of Fish Biology.* **61**, 1410-1422.

Clark, T. D., Taylor, B. D., Seymour, R. S., Ellis, D., Buchanan, J., Fitzgibbon, Q. P. and Frappell, P. B. (2008). Moving with the beat: heart rate and visceral temperature of free-swimming and feeding bluefin tuna. *Proc. R. Soc. B.* **275**, 2841-2850.

Clarke, S. and Ham, J. (2002). Southern bluefin tuna surrogate research. South Australian Research and Development Institute, South Australia. 71pps.

Clarke, S., Smart, A., van Barneveld, R. and Carter, C. (1997). The development and optimisation of manufactured feeds for farmed southern bluefin tuna. *Austasia Aquaculture.* **11 (3)**, 59-62.

Collette, B. B. (1978). Adaptations and Systematics of the Mackerels and Tunas. In: G.D. Sharp and A.E. Brizon (Eds.), The Physiological Ecology of Tunas, Academic Press, New York, pp 7–40.

Collette, B. B. and Nauen, C. E. (1983). FAO Species Catalogue. Vol. 2. Scombrids of the World. An Annotated and Illustrated Catalogue of Tunas, Mackerels, Bonitos and Related Species Known to Date. Rome: Food and Agriculture Organization of the United Nations.

Collette, B. B., Reeb, C. and Block, B. A. (2001). Systematics of the tunas and mackerels. In: B. A. Block and E. D. Stevens (Eds.), In: B. A. Block and E. D. Stevens (Eds.), Tuna physiology, ecology, and evolution. Academic Press, San Diego, pp. 5-30.

Cooke, S. J., Phillip D. P., Schreer, J. F. and McKinley, R. S. (2000). Locomotory impairment of nesting largemouth bass following catch-and-release angling. *North American Journal of Fisheries Management.* **20-4**, 968–977.

Cooke, S. J., Schreer, J. F., Wahl, D. H. and Phillip, D. P. (2002). Physiological impacts of catch-and release angling practices on largemouth bass and smallmouth bass. *Black bass 2000: Ecology, Conservation, and Management of Black Bass in North America, St Louis, MO (USA), 21 – 24 Aug 2000.*

Cribb, T. H., Adlard, R. D., Hayward, C. J., Bott, N. J., Ellis, D., Evans, D., Nowak, B. F. (2011). The life cycle of *Cardicola forsteri* (Trematoda: Aporocotylidae), a pathogen of ranched southern bluefin tuna, *Thunnus maccoyii*. *International Journal for Parasitology*. doi: 10.1016/j.ijpara.2011.03.011.

Crozier, W and Kennedy, G. (2002). Impact of tagging with coded wire tags on marine survival of wild Atlantic salmon (*Salmo salar* L.) migrating from the R. Bush. *Northern Ireland Fisheries Research*. **59** Vol 2, 209-215.

Dennis, M. M, Landos, M. and D'Antignana, T. (2011). Case-control study of epidemic mortality and *Cardicola forsteri*-associated disease in farmed southern bluefin tuna (*Thunnus maccoyii*) of South Australia. *Vet Pathol.* **48**, 846–855.

Dewar, H. and Graham, J. B. (1994). Studies of tropical tuna swimming performance in a large water tunnel - Energetics. *Journal of Experimental Biology.* **192**, 13-31.

Dewar, H. and Graham, J. B. and Brill, R. W. (1994). Studies of tropical tuna swimming performance in a large water tunnel. II. *Journal of Experimental Biology.* **192**, 33-44.

Dickson, K. A. (1994). Tunas as small as 207mm fork length can elevate muscle temperature significantly above ambient temperature. *Journal of Experimental Biology.* **190**, 79–93.

Dickson, K. A. (1996). Locomotor Muscle of High-performance Fishes: What do Comparisons of Tunas with Ectothermic Sister Taxa Reveal? *Comp. Biochem. Physiol.* **Vol 113A 1**, 39-49.

Dickson, K. A. and Graham, J. B. (2004). Evolution and consequences of endothermy in fishes. *Physiological and Biochemical Zoology.* **77**, 998-1018.

Dickson, K. A., Johnson, N., Donley, J. M., Hoskinson, J. A., Hansen, M. W. and Tessier, J. D. (2000). Ontogenetic changes in characteristics required for endothermy in juvenile black skipjack tuna (*Euthynnus lineatus*). *Journal of Experimental Biology.* **203**, 3077-3087.

Dizon, A. E. and Brill, R. W. (1979a). Thermoregulation in the yellowfin tuna. *Thunnus albacares*. *Physiological Zoology.* **52**, 581-593.

Dizon, A. E. and Brill, R. W. (1979b). Thermoregulation in tunas. *American Zoologist.* **19**, 249-265.

Dizon, A. E., Brill, R. W. and Yuen, H. S. H. (1978). Correlations between environment, physiology, and activity and the effects on thermoregulation in skipjack tuna. In: G. D. Sharp and A. E. Dizon (Eds.), The Physiological Ecology of Tunas. Academic Press, San Diego, pp. 233-259.

Econsearch (2009). Viewed 20 March 2012, http://www.econsearch.com.au/

Ellis, D. and Rough, K. (2005). Quality and nutritional evaluation of baitfish used for SBT farming (including baitfish profiles), Aquafin CRC, Adelaide, pp. 1-39.

Eriksson, L. O. and Alanärä, A. (1992). Timing of feeding behaviour in salmonids. In: J.E. Thorpe and F.A. Huntingford (Eds.), The Importance of Feeding Behaviour for the Efficient Culture of Salmonid Fishes World Aquaculture Society, Baton Rouge, Louisiana, pp. 41 - 48.

FAO Fishery Statistics. Viewed on 18 July, 2011, www.fao.org

Fitzgibbon, Q. P., Baudinette, R. V., Musgrove, R. J. and Seymour, R. S. (2006). The routine metabolic rate of southern bluefin tuna (*Thunnus maccoyii*). *Comparative Biochemistry and Physiology* **A**. doi:10.1016/j.cbpa.2006.08.046.

Fitzgibbon, Q. P., Seymour, R. S., Ellis, D. and Buchanan, J. (2007). The energetic consequence of specific dynamic action in southern bluefin tuna (*Thunnus maccoyii*). *Journal of Experimental Biology*. **210**, 290-298.

Fitzgibbon, Q. P., Frappell, P., Clark, T., Buchanan, J., Ellis, D., Carter, C., Musgrove, R. Carragher, J. and Seymour, R. (2008). Activity metabolism in live-held southern bluefin tuna (*Thunnus maccoyii*). *Aquafin CRC Project 1 A.7 (2), (FRDC Project No. 2005/200)* Printed by SARDI, South Australia, 123pps.

Fudge, D. S. and Stevens, E. D. (1996). The visceral *retia mirabilia* of tuna and sharks: an annotated translation and discussion of the Eschricht & Muller 1835 paper and related papers. *Guelph Ichthyology Reviews.* **4**, 1-53.

Fudge, D. S., Stevens, E. D. and Ballantyne, J. S. (1997). Enzyme adaptation along a heterothermic tissue: the visceral retia mirabile of the bluefin tuna. *A. J. Physiol.* **272**, R1834–R1840.

Geen, G. and Nayer, M. (1989). Individual transferable quotas and the southern bluefin tuna fishery, economic paper. Occasional paper 105, Project 62336. Australian Publishing and Printing Co, Fyshwick, A.C.T. 48pps.

Geurden, I. Gondouin, E., Rimbach, M., Koppe, W., Kaushik, S. and Boujard, T. (2006). The evaluation of energy intake adjustments and preferences in juvenile rainbow trout fed increasing amounts of lipid. *Physiology and Behaviour.* **88**, 325-332.

Glencross, B. D., van Barneveld, R. J., Carter, C. G. and Clarke, S. M. (1999). On the path to a manufactured feed for farmed southern bluefin tuna. *World Aquaculture Magazine.* **30 (3)**, 42-47.

Glencross, B. D., Clarke, S., Buchanan, J., Carter, C. G. and van Barneveld, R. J. (2002). Temporal growth patterns of farmed juvenile southern bluefin tuna, *Thunnus maccoyii* (Castelnau) fed moist pellets. *Journal of the World Aquaculture Society.* **33**, 138-145.

Gordon, R., Bayly, G. and Foster, C. (2006a). Commercialisation trials for a manufactured feed. *Aquafin CRC Project 1 A.3, (FRDC Project No. 2001/201),* Printed by SARDI, South Australia, 123pps.

Gordon, R., Bayly, G. and Foster, C. (2006b). Industry report on commercial pellet trial on southern bluefin tuna (*Thunnus maccoyii*) conducted by Australian Tuna Fisheries and Skretting Australia. *Commercial in confidence.* Printed by Skretting Australia, Cambridge, Tasmania, 43pps.

Graham, J. B. and Dickson, K. A. (2000). The evolution of thunniform locomotion and heat conservation in scombrid fishes: New insights based on the morphology of *Allothunnus fallai*. *Zoological Journal of the Linnean Society.* **129**, 419-466.

Graham, J. B. and Dickson, K. A. (2001). Anatomical and physiological specializations for endothermy. In: B. A. Block and E. D. Stevens (Eds.), Tuna physiology, ecology, and evolution. Academic Press, San Diego, pp. 121-165.

Guinet, C., Domenici, P., de Stephanis, R., Barrett-Lennard, L. Ford, J. K. B. and Verbough, P. (2007). Killer whale predation on bluefin tuna: exploring the hypothesis of the endurance-exhaustion technique. *Mar Ecol Prog Ser.* **347**, 111-119.

Gunn, J. and Block, B. A. (2001). Advances in acoustic, archival, and satellite tagging of tunas. In: B. A. Block and E. D. Stevens (Eds.), Tuna physiology, ecology, and evolution. Academic Press, San Diego, pp. 167-224.

Gunn, J. S., Polacheck, T., Davis, T. L. O., Sherlock, M. and Betlehem, A. (1994). The development and use of archival tags for studying the migration, behaviour and physiology of Southern Bluefin Tuna, with an assessment of the potential for transfer

of the technology to groundfish research. *Proc I.C.E.S. Mini Symposium on Fish Migration.* **21**, 23pp.

Gunn, J., Hartog, J. and Rough, K. (2001). The relationship between food intake and visceral warming in southern bluefin tuna (*Thunnus maccoyii*). In: J. R. Sibert and J. L. Nielsen (Eds.), Electronic Tagging and Tracking in Marine Fisheries vol. 1. Academic Publishers, Dordrecht: Kluwer ,pp. 1009-130.

Gunn, J., Hartog, J. and Rough, K. (2002). Experimental analyses of the effects of ration and feeding frequency on the thermodynamics, energetics, growth and condition of farmed Southern Bluefin Tuna. *Fisheries Research and Development Corporation Final Report*, Project 97/363. 176pps

Hamre, K., Kolas, K., Sandnes, K., Julshammn, K. and Kiessling, A. (2001). Feed intake and absorption of lipid oxidation products in Atlantic salmon (*Salmo salar*) fed diets coated with oxidised fish oil. *Fish Physiology and Biochemistry.* **25-3**, 209-219.

Haskard, K., Su, S., Buchanan, J., Madigan, S. and Clarke, S. (2001). Power tables for Southern Bluefin Tuna *(Thunnus maccoyii)* feed development experiments. *BiometricsSA,* South Australian Research and Development Institute, Aquatic Sciences. Adelaide SA., 21pps.

Hayes, E. A. (1997). A Review of the Southern Bluefin Tuna Fishery: implications for ecologically sustainable management. *A TRAFFIC Oceania Report,* 34pps.

Hayward, C. J., Ellis, D., Foote, D., Wilkinson, R. J. and Crosbie, P. B. B. (2010). Concurrent epizootic hyperinfections of sea lice (predominantly *Caligus chiastos*) and blood flukes (*Cardicola forsteri*) in ranched southern bluefin tuna. *Veterinary Parasitology.* **173 1-2**, 107–115.

Helland, S. J., Grisdale-Helland, B. and Berge, G. M. (1997). Feed intake and growth of Atlantic halibut (*Hippoglossus hippoglossus*) fed combinations of pellet sizes. *Aquaculture.* **156**, 1-8.

Holland, K. N., Brill, R. W., Chang, R. K. C., Sibert, J. R. and Fournier, D. A. (1992). Physiological and behavioural thermoregulation in bigeye tuna (*Thunnus obesus*). *Nature.* **358**, 410-412.

Hone, P., Vandepeer, M. and Clarke, S. (1996). Environmental Site Assessment for proposed tuna aquaculture zones – Rabbit Island, Boston Island and Taylor Island. *South Australian Research and Development Institute*, West Beach. 37pps

Hutchison, W., Black, I. and Buchanan, J. (2008). Assessment of alternative platforms for southern bluefin tuna research, Adelaide: Aquafin CRC. pp. 1-57.

Itoh, T., Kemps, H. and Totterdell, J. (2011). Diet of young southern bluefin tuna (*Thunnus maccoyii*) in the southwestern coastal waters of Australia in summer. *Fish Sci.* **77**, 337-344.

Itoh, T., Tsuji, S. and Nitta, A. (2003). Swimming depth, ambient water temperature preference, and feeding frequency of young Pacific bluefin tuna (*Thunnus orientalis*) determined with archival tags. *Fishery Bulletin.* **101**, 535-544.

Jarboe, H. H. and Grant, W. J. (1996). Effects of feeding time and frequency on growth of channel catfish, *Ictalurus punctatus*, in closed recirculating raceway systems. *Journal of the World Aquaculture Society.* **27**, 235-239.

Jeffriess, B. (1999). Growout of Southern Bluefin Tuna. *Fisheries Research and Development Corporation.* Project No. 91/56*,* 112 pages.

Jobling, M. (1994). Fish bioenergetics. Chapman and Hall, London.

Jobling, M. (2001). Feed composition and analysis. In: D. Houlihan, T. Boujard and M. Jobling (Eds.), Food intake in fish. Blackwell Science, Iowa, USA, pp. 1 - 21.

Jobling, M. and Baardvik, B. M. (1991). Patterns of growth of maturing and immature Arctic charr, *Salvelinus alpinus* in a hatchery population. *Aquaculture,* **94**, 343-354.

Jobling, M. and Johansen, S. J. S. (1999). The lipostat, hyperphagia and catch-up growth. *Aquaculture Research*. **30**, 473-478.

Jobling, M. and Koskela, J. (1999). The lipostat, hyperphagia and catch-up growth. *Aqua. Research.* **30**, 473-478.

Jobling, M. & Miglavs, I. (1993). The size of lipid depots – a factor contributing to the control of food intake in Arctic charr, *Salvelinus alpinus*? *Journal of Fish Biology*. **43**, 487-489.

Jobling, M., Coves, D., Damsgard, B., Kristiansen, H. R., Koskela, J., Petursdottir, T. E., Kadri, S. and Gudmundsson, O. (2001). Techniques for measuring feed intake. In: D. Houlihan, T. Boujard and M. Jobling (Eds.), Food intake in fish. Blackwell Science, Iowa, USA, pp. 49-88.

Johansen, S. J. S., Ekli, M., Stagnes, B. and Jobling, M. (2001). Weight gain and lipid deposition in Atlantic salmon, *Salmo salar,* during compensatory growth: evidence for lipostatic regulation? *Aquaculture Research.* **32**, 963-974.

Johansen, S. J. S., Ekli, M. and Jobling, M. (2002). Is there lipostatic regulation of feed intake in Atlantic salmon *Salmo salar L.*? *Aquaculture Research.* **33**, 515-524.

Johansen, S .J. S., Sveier, H. and Jobling, M. (2003). Lipostatic regulation of feed intake in Atlantic salmon *Salmo salar* L. defending adiposity at the expense of growth? *Aquaculture Research*. **34,** 317-331.

Jones, R. E., Petrell, R. J. and Pauly, D. (2009). Using modified length-weight relationships to assess the condition of fish. *Aquacultural Engineering.* **20**, 261-276.

Jørgenson, E. H. and Jobling, M. (1993). Feeding in darkness eliminates density-dependent growth suppression in Arctic Charr (*Salvelinus alpines*). *Aquaculture International.* **1**, 90-93.

Juell, J.-E., Bjordal, Å., Fernö, A. and Huse, I. (1994). Effect of feeding intensity on food intake and growth of Atlantic salmon, *Salmo salar* L., in sea cages. *Aquaculture and Fisheries Management.* **22**, 377-378.

Kadri, M. H., Metcalfe, N. B., Huntingford, F. A. and Thorpe, J. E. (1997). Daily feed rhythms in Atlantic salmon II: size-related variation in feeding patterns of post-smolts under constant environmental conditions. *Journal of Fish Biology.* **50,** 273–279.

Katavić, I., Tícina, V. and Franičevič, V. (2003). Rearing of small bluefin tunas (*Thunnus thynnus* L.) in the Adriatic Sea – preliminary study. In: Bridges, C.R., Gordin, H., Garcia, A. (Eds.), Proceedings of the Symposium on Domestication of the Bluefin Tuna, *Thunnus thynnus thynnus,* 3-8 February 2002. Cartagena, Spain. Cah. *Options Mediterr*. vol. **60**, 95-99.

Keen, J. E., Aota, S., Brill, R. W., Farrell, A. P. and Randall, D. J. (1995). Cholinergic and adrenergic regulation of heart rate and ventral aortic pressure in two species of tropical tunas, *Katsuwonus pelamis* and *Thunnus albacares*. *Can. J. Zool.* **73**, 1681-1688.

Keiffer, J. D., Kubacki, M. R., Phelan, F. J. S., Phillip D. P. and Tufts, B. L. (1995). Effects of catch and release angling on nesting smallmouth bass. *Transactions of the American Fisheries Society.* **124**, 70-76.

Kennedy, G. C. (1953). The role of depot fat in the hypothalamic control of food intake in the rat. *Proceedings of the Royal Society B*. **140**, 578-592.

Kerdchuen, N. and Legendre, M. (1991). Effects of frequency and period of feeding on growth rate and feed efficiency of an African catfish, *Hetero longifilis* (Teleostei, Clariidae). *Aquatic Living Resources.* **4.4**, 241-248.

Kitagawa, T., Nakata, H., Kimura, S., Itoh, T., Tsuji, S. and Nitta, A. (2000). Effect of ambient temperature on the vertical distribution and movement of Pacific bluefin tuna *Thunnus thynnus orientalis*. *Marine Ecology Progress Series.* **206**, 251-260.

Kitagawa, T., Nakata, H., Kimura, S. and Tsuji, S. (2001). Thermoconservation mechanisms inferred from peritoneal cavity temperature in free-swimming Pacific bluefin tuna *Thunnus thynnus orientalis*. *Marine Ecology Progress Series.* **220**, 253-263.

Kitagawa, T., Kimura, S., Nakada, M. and Yamada, H. (2004). Diving behaviour of immature, feeding Pacific bluefin tuna (*Thunnus thynnus orientalis*) in relation to season and area: the East China Sea and the Kuroshio-Oyashio transition region. *Fisheries Oceanography.* **13**, 161-180.

Kitagawa, T., Kimura, S., Nakata, H. and Yamada, H. (2006). Thermal adaptation of Pacific bluefin tuna *Thunnus orientalis* to temperate waters. *Fisheries Science.* **72**, 149-156.

Kitagawa, T., Kimura, S., Nakata, H. and Yamada, H. (2007). Why do young Pacific bluefin tuna repeatedly dive to depths through the thermocline? *Fisheries Science.* **73**, 98-106.

Korsmeyer, K. E. and Dewar, H. (2001). Tuna metabolism and energetics. In: B. A. Block and E. D. Stevens (Eds.), Tuna physiology, ecology, and evolution. Academic Press, San Diego, pp. 35-78.

Korsmeyer, K. E., Dewar, H., Lai, N. C. and Graham, J. B. (1996). The aerobic capacity of tunas: Adaptation for multiple metabolic demands. *Comparative Biochemistry and Physiology.* **113A**, 17-24.

Korsmeyer, K. E., Lai, N. C., Shadwick, R. E., and Graham, J. B. (1997a). Heart rate and stroke volume contributions to cardiac output in swimming yellowfin: Responses to exercise and temperature. *Journal of Experimental Biology*. **200,** 1975-1986.

Korsmeyer, K. E., Lai, N. C., Shadwick, R. E., and Graham, J. B. (1997b). Oxygen transport and cardiovascular responses to exercise in the yellowfin tuna *Thunnus albacares. Journal of Experimental Biology.* **200,** 1987-1997.

Kousoulaki, K., Miliou, E., Apostolopoulou, M. and Alexis, M. N. (2007). Effect of feeding intensity and feed composition on nutrient digestibility and production performance of common Pandora (*Pagellus erythrinus*) in sea cages. *Aquaculture.* **272,** 514-527.

Lagler, K. F., Bardach, J. E., Miller, R. R. and May Passino, D. R. (1977). *Ichthyology. Second Edition*. John Wiley and Sons, Brisbane.506pps.

Laurs, R. M., Yuen, H. S. H., and Johnson, J. H. (1977). Small scale movements of albacore, *Thunnus alalunga*, in relation to ocean features as indicated by ultrasonic tracking and oceanographic sampling. *Fish. Bull. U.S.* **75**, 347–357.

Lazo, J. P. and Davis, D. A. (2000). Ingredient and feed evaluation. In: R. Stickney (Ed), Encyclopaedia of Aquaculture. John Wiley and Sons, Canada, pp 453-464.

Leatherland, J. F., Farbridge, K. J., and Boujard, T. (1992). Lunar and semi-lunar rhythms in fishes. In: M.A. Ali (Ed.), Rhythms in Fishes. Plenum Press, New York, pp. 83-107.

Lee, S. -M. and Pham, M. A. (2010). Effects of feeding frequency and feed type on the growth, feed utilization and body composition of juvenile olive flounder *Paralichthys olivaceus*. *Aquaculture Research.* **41.9**, 166-171.

Lee, S-M., Seo, C-H. and Cho, Y-S. (1998). Growth of the juvenile olive flounder (*Paralichthys olivaceus*) fed the diets at different feeding frequencies. *J. Korean Fish. Soc*. **32**, 18-21.

Lee, S. -M., Kim, D. -J. and Cho, S. H. (2002). Effects of dietary protein and lipid level on growth and body composition of juvenile ayu (*Plecoglossus altivelis*) reared in seawater. *Aquaculture Nutrition.* **8**, 53-58.

Liu, Fu-G. and Liao, I. C. (1999). Effect of feeding regimen on the food consumption, growth, and body composition in hybrid striped bass *Morone saxatilis* x *M. chrysops. Fisheries science. Tokyo*. **65**, 513-519.

Liu, Y. H., Sakamoto, H., Adachi, M., Zhao, S. G., Ukai, W., Hashimoto, E., Hareyama, M., Ishida, T., Imai, K. and Shinomura, Y. (2012). Heat stress activates ER stress signals which suppress the heat shock response, an effect occurring preferentially in the cortex in rats. *Molecular Biology Reports.* **39**, 87-93.

Loftus, A.J., Taylor, W. W., and Keller, M. (1988). An evaluation of lake trout (*Salvelinus namaycush*) hooking mortality in the upper great lakes. *Can. J. Fish. Aquat.* Sci. **45**, 1473-1479.

Loftus, T. M. (1999). An adipocyte-central nervous system regulatory loop in the control of adipose homeostasis. *Seminars in Cell and Developmental Biology*. **10**, 11-18.

Madrid, J. A., Boujard, T. and Sanchez-Vazquez, F. J. (2001). Feeding Rhythms. In: D. Houlihan, T. Boujard and M. Jobling (Eds.), Food intake in fish. Blackwell Science, Iowa, USA, pp. 189-216.

Magnuson, J. J. (1969). Digestion and food consumption by skipjack tuna (*Katsuwonus pelamis*). *Transactions of the American Fisheries Society.* **98**, 379-392.

Magnuson, J. J. (1978). Locomotion by scombrid fishes: Hydromechanics, morphology, and behaviour. In: W. Hoar and D.J. Randall (Eds.), Fish Physiology Vol. 7, Academic Press, New York, pp 239–313.

Malte, H., Larsen, C., Musyl, M. and Brill, R. (2007). Differential heating and cooling rates in bigeye tuna (*Thunnus obesus* Lowe): a model of non-steady state heat exchange. *Journal of Experimental Biology*. **210**, 2618-2626.

Marcinek, D. J., Blackwell, S. B., Dewar, H., Freund, E. V., Farwell, C., Dau, D., Seitz, A. C. and Block, B. A. (2001). Depth and muscle temperature of Pacific bluefin tuna examined with acoustic and pop-up satellite archival tags. *Marine Biology.* **138**, 869-885.

Martins, D. A., Valente, L. M. P., and Lall, S. P. (2009). Apparent digestibility of lipid and fatty acids in fish oil, poultry fat and vegetable oil diets by Atlantic halibut, *Hippoglossus hippoglossus L*. *Aquaculture.* **294**, 132-137.

Mazlan, A. G. and Grove, D. J. (2003). Gastric digestion and nutrient absorption along the alimentary tract of whiting (*Merlangius merlangus*) fed on natural prey. *Journal of Applied Ichthyology.* **19.4**, 229-238.

McCarthy, I. D., Carter, C. G. and Houlihan, D. F. (1993). Individual variation in consumption in rainbow trout measured using radiography. In: S. J. Kaushik and P. Luquet (Eds.), Fish Nutrition in Practice. INRA Editions, Paris, pp. 85-88.

McLeay, L. J., Jones, G. K. and Ward, T. M. (2002). *National Strategy for the Survival of Released Line-caught Fish: A Review of Research and Fishery Information*. Fisheries Research and Development Corporation Project 2001/101. Henley Beach, South Australia. South Australian Research and Development Institute, 121 pp.

Meka, J. M. (2004). The influence of hook type, angler experience, and fish size on injury rates and the duration of capture in an Alaskan catch-and-release rainbow trout fishery. *North American Journal of Fisheries Management.* **24**, 1299–1311.

Meka, J. M. and Margraf, F. J. (2007). Using a bioenergetic model to assess growth reduction from catch-and-release fishing and hooking injury in rainbow trout, *Oncorhynchus mykiss. Fisheries management and Ecology.* **14**, 131–139.

Metcalfe, N. B. and Thorpe, J. E. (1992). Anorexia and defended energy levels in over-wintering juvenile salmon. *Journal of Animal Ecology*. **61**, 175-181.

Millar, R. B. and Anderson, M. J. (2004). Remedies for pseudoreplication. *Fisheries Research.* **70**, 397-407.

Montague, P., Downes, E. and Ham, J. (2008). Achievements and impacts (2001-2008), the cooperative research centre for the sustainable aquaculture of finfish. Stallard and Potter, Adelaide, 45pps.

Morales, A. E., Cardenete, G., Sanz, A. and de la Higuera, M. (1999). Re-evaluation of crude fibre and acid-insoluble ash as inert markers, alternative to chromic oxide, in digestibility studies with rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. **179**, 71–79.

Musgrove, R. and Fitzgibbon, Q. (2006). Activity metabolism in live-held southern bluefin tuna (*Thunnus maccoyii*), Aquafin CRC, Adelaide, pp. 1-57.

Nikki, J., Pirhonen, J., Jobling, M. and Karjalainen, J. (2004). Compensatory growth in juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum), held individually. *Aquaculture.* **235**, 285-296.

Noël, O. and Le Bail., P.-Y. (1997). Does cyclicity of growth rate in rainbow trout exist? *Journal of Fish Biology.* **51**, 634-642.

Nowak, B. F., Evans, D., Ellis, D., Wilkinson, R., Porter, M., Crosbie, P., Leef, M. and Hayward, C. (2010). Improving husbandry and performance of southern bluefin tuna through better understanding of the relationship between fish stress and health. Aquafin CRC, Adelaide, pp. 1-79.

Olson, R. J. and Boggs, C. H. (1986). Apex predation by yellowfin tuna (*Thunnus albacares*): Independent estimates from gastric evacuation and stomach contents, bioenergetics, and caesium concentrations. *Canadian Journal of Fisheries and Aquatic Sciences.* **43**, 1760-1775.

Olson, R. J. and Scholey, V. P. (1990). Captive tunas in a tropical marine research laboratory: Growth of later-larval and early juvenile black skipjack *Euthynnus lineatus. Fish. Bull. U.S.* **88**, 821–828.

Øverli, O., Winberg, S., Damsgard, B. and Jobling, M. (1998). Food intake and spontaneous swimming activity in Arctic charr (*Salvelinus alpinus*): role of brain serotonergic activity and social interactions. *Canadian Journal of Zoology.* **76**, 1366–1370.

Parsons, G. R. and Carlson, J. K. (1998). Physiological and behavioural response to hypoxia in the bonnethead shark, *Syhyrna tiburo*: routine swimming and respiratory regulation. *Fish Physiology and Biochemistry.* **19**, 189-196.

Patterson, H., Begg, G., Curtotti, R. and Vieira, S. (2009). Southern Bluefin Tuna Fishery. In: D.T. Wilson, R. Curtotti and G. A. Begg (Eds.), Fishery status reports 2009 – Status of Fish Stocks and Fisheries Managed by the Australian Government. Canberra: Union offset printers, Canberra, A.C.T., pp. 422-433.

Patterson, H., Begg, G., and Hormis, M. (2010). Southern Bluefin Tuna Fishery. In: J. Woodhams, I. Stobutzki, S. Vieira, R. Curtotti and G. Begg (Eds.), Fishery status reports 2009 – Status of Fish Stocks and Fisheries Managed by the Australian Government. Canberra: Union offset printers, Canberra, A.C.T., pp. 359-367.

Petrusevics, P. (1993). Assessment of the carrying capacity of Boston Bay South Australia with a view towards maximising the southern bluefin tuna resource. *Fisheries Research and Development Corporation Final Report*, Project 93/169. 31pps

Patterson, H., Begg, G., and Hormis, M. (2010). Southern Bluefin Tuna Fishery. In: J. Woodhams, I. Stobutzki, S. Vieira, R. Curtotti and G. Begg (Eds.), Fishery status reports 2009 – Status of Fish Stocks and Fisheries Managed by the Australian Government. Canberra: Union offset printers, Canberra, A.C.T., pp. 359-367.

Phillip, D. P., Toline, C. A., Kubacacki, M. R., Philipp, D. B. F. and Phelan, F. J. S. (1997). The impact of catch-and-release angling on the reproductive success of smallmouth bass and largemouth bass. *North American Journal of Fisheries.* **17**, 557-567.

PIRSA. (2012). Aquaculture industry/tuna, viewed 6 April 2012. http://www.pir.sa.gov.au/aquaculture/aquaculture_industry/tuna

Plevin, J.E. (2000). MFV Tacoma and the Haldane family, viewed 18 January 2012. http://tacoma.org.au/documents/Tacoma_Haldane_History.pdf

Polacheck, T., Eveson, J. P. and Laslett, G. M. (2004). Increase in growth rates of southern bluefin tuna (*Thunnus maccoyii*) over four decades: 1960 to 2000. *Can. J. Fish. Aquat. Sci.* **61**, 307-322.

Pope, K. L. and Wilde, G. R. (2004). Effect of catch-and-release angling on growth of largemouth bass, *Micropterus salmoides*. *Fisheries Management and Ecology.* **11**, 39–44.

Pope, K. L., Wilde, G. R. and Knabe, D. W. (2007). Effect of catch-and-release angling on growth and survival of rainbow trout, *Oncorhynchus mykiss. Fisheries Management and Ecology.* **14**, 115-121.

Power, M. L. and Schulkin, J. (2008). Anticipatory physiological regulation in feeding biology: Cephalic phase responses. *Appetite.* **50**, 194-206.

Prosser, C. L. (1973). Temperature. Chapter 9. In: C.L. Prosser (Ed.), Comparative Animal Physiology. W.B. Saunders, Philadelphia. pp 1011

Purser, G. J. and Chen, W.-M. (2001). The effect of meal size and meal duration on food anticipatory activity in greenback flounder. *Journal of Fish Biology.* **58**, 188-200.

Quinn, S. P. (1989). Recapture rates of voluntarily released largemouth bass. *North American Journal of Fisheries Management.* **9**, 86–91.

Ranta, T. and Pirhonen, J. (2006). Effect of tank size on food intake and growth in individually held juvenile rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research*. **37**, 1381–1385.

Reddy, P. K., Leatherland, J. F., Khan, M. N. and Boujard, T. (1994). Effect of the daily meal time on the growth of rainbow trout fed different ration levels. *Aquaculture International.* **2**, 1–15.

Riley, J. and Edwards, P. (1998). Statistical aspects of aquaculture research: pond variability and pseudoreplication. *Aquaculture Research.* **29**, 281-288.

Runcie, R. M., Dewar, H., Hawn, D. R., Frank, L. R. and Dickson, K. A. (2009). Evidence for cranial endothermy in the opah (*Lampris guttatus*). *Journal of Experimental Biology.* **212**, 461-470.

Ruohonen, K., Kettunen, J. and King, J. (2001). Experimental design in feeding experiments. In: D. Houlihan, T. Boujard and M. Jobling (Eds.), Food intake in fish. Blackwell Science, Iowa, USA, pp. 88-108.

Ruohonen, K., Simpson, S. J. and Raubenheimer, D. (2007). A new approach to diet optimisation: A re-analysis using European whitefish (*Coregonus lavaetus*). *Aquaculture.* **267**, 147-156.

Saether, B. S. and Jobling, M. (2001). Fat content in turbot feed: influence on feed intake, growth and body composition. *Aquaculture Research.* **32**, 451-458.

Salama, A. (2008). Effects of different feeding frequency on the growth, survival and feed conversion ratio of the Asian sea bass *Lates calcarifer* juveniles reared under hypersaline seawater of the Red Sea. *Aquaculture Research.* **39.6**, 561-567.

Sánchez-Vázquez, F. J. and Madrid, J. A. (2001). Feeding anticipatory activity. In: D. Houlihan, T. Boujard and M. Jobling (Eds.), Food intake in fish. Blackwell Science, Iowa, USA., pp. 216-232.

Sánchez-Vázquez, F. J., Madrid, J. A., Zamora, S. and Tabata, M. (1997). Feeding entrainment of locomotor activity rhythms in the goldfish is mediated by a feeding-entrainable circadian oscillator. *Journal of Comparative Physiology*. **181A**, 121–132.

Schaefer, K. M. (1984). Swimming performance, body temperatures, and gastric evacuation times of black skipjack, *Euthynnus lineatus*. *Copeia.* **1984**, 1001-1004.

Schaefer, K. M., Fuller, D. W. and Block, B. A. (2007). Movements, behaviour, and habitat utilisation of yellowfin tuna (*Thunnus albacares*) in the north-eastern Pacific Ocean, ascertained through archival tag data. *Marine Biology.* **152**, 503-525.

Schisler, G. J. and Bergersen, E. P. (1996). Post-release hooking mortality of rainbow trout caught on scented artificial baits. *N. Am. J. Fish. Man*. **16**, 570-578.

Sepulveda, C. A., Dickson, K. A. and Graham, J. B. (2007). Cranial endothermy and a putative brain heater in the most basal tuna species, *Allothunnus fallai*. *Journal of Fish Biology.* **70**, 1720–1733.

Serventy, D. L. (1956). The Southern Bluefin Tuna, *Thunnus thynnus maccoyii* (Castelnau), in Australian Waters. *Aust Journal of Marine and Freshwater Research.* **7**, 1-43.

Shearer, K. D., Silverstein, J. T. and Plisetskaya, E. M. (1997). Role of adiposity in food intake control of juvenile Chinook salmon (*Oncorhynchus tshawytscha*). *Comparative Biochemistry and Physiology A*. **118**, 1209-1215.

Sigurgisladottir, S. S., Lall, S. P., Parrish, C. C. and Ackman, R. G. (1992). Cholestane as a digestibility marker in the absorption of polyunsaturated fatty acid ethyl esters in Atlantic salmon. *Lipids.* **27.6**, 418-424.

Silverstein, J. T. and Plisetskaya E. M. (2000). The effects of NPY and insulin on food intake regulation in fish. *American Zoologist*. **40**, 269-308.

Silverstein, J. T., Shearer, K.D., Dickhoff, W. W. and Plisetskaya, E. M. (1999). Regulation of nutrient and energy balance in salmon. *Aquaculture*. **177**, 161-169.

Smith, I. P., Metcalfe, N. B., Huntingford, F. A. and Kadri, S. (1993). Daily and seasonal patterns in the feeding behaviour of Atlantic salmon (*Salmo salar* L.) in a sea cage. *Aquaculture.* **117**, 165-178.

Spieler, R. E. (1992). Feeding entrained circadian rhythms in fishes. In: M.A. Ali (Ed.), Rhythms in fishes. Plennum Press, New York, pp. 137-148.

Stevens, E. D. and Carey, F. G. (1981). One why of the warmth of warm-bodied fish. *American Journal of Physiology*. **240**, R151-R155.

Stevens, E. D. and McLeese, J. M. (1984). Why bluefin tuna have warm tummies: temperature effect on trypsin and chymotripsin. *American Journal of Physiology.* **246**, R487-R494.

Stevens, E. D., and Neill, W. H. (1978). Body temperature relations of tuna, especially skipjack. In: G. D. Sharp and A. E. Dizon (Eds.), The Physiological Ecology of Tunas. Academic Press, San Diego, pp. 316-360.

Stevens, E. D., Kanwisher, J. W. and Carey, F. G. (2000). Muscle temperature in free-swimming giant Atlantic bluefin tuna (*Thunnus thynnus* L.). *Journal of Thermal Biology.* **25**, 419-423.

Stokesbury, J. W., Neilson, J. D., Susko, E. and Cooke, S. J. (2011). Estimating mortality of Atlantic bluefin tuna (Thunnus thynnus) in an experimental recreational catch-and-release fishery. *Biological Conservation.* **144-11**, 2684-2691.

Storebakken, E. T. and Austreng, E. (1988). Feed intake measurements in fish using radioactive isotopes. II. Experiments with Atlantic salmon and rainbow trout in sea-pens. *Aquaculture.* **70-3**, 277-288.

Sugiura, S.H. (2000). Digestibility. In R. Stickney (Ed), Encyclopaedia of Aquaculture. John Wiley and Sons, Canada, pp 209-218.

Sumpton, W., Mayer, D., Brown, I., Sawynok, B., McLennan, M., Butcher, A. and Kirkwood, J (2008). Investigation of movement and factors influencing post-release survival of line-caught coral reef fish using recreational tag-recapture data. *Fisheries Research*. **92**, 189–195.

Thodesen, J., Grisdale-Helland, B. and Gjerde, B. (1999). Feed intake, growth and feed utilisation of offspring from wild and selected Atlantic salmon (*Salmo salar)*. *Aquaculture.* **180 3-4**, 237-246.

Thomas, P. (2007). Maximising the control of quality in farmed SBT. Aquafin CRC, Adelaide, pp. 1-215.

Thomas, P., Thomas, M., Schuller, K. and D'Antignana, T. (2009). Application of the use of dietary supplements for improving flesh quality attributes of farmed SBT. Aquafin CRC, Adelaide, pp. 1-167.

Tičina, V., Katavić, I. and Grubišić, L. (2007). Growth indices of small northern bluefin tuna (*Thunnus thynnus,* L.) in growth-out rearing cages). *Aquaculture.* **269**, 538-543.

Tuerker, A., and Yildirim, Oe. (2011). The effect of feeding frequency on growth performance and body composition in juvenile rainbow trout (*Oncorhynchus mykiss*) reared in cold seawater. *African Journal of Biotechnology*. **10** , 79-84.

van Barneveld R. and Ellis, D. C. (2007). Practical nutrition of farmed Southern Bluefin Tuna *Thunnus maccoyii.* Aquafin CRC, Adelaide. Commercial in confidence.

van Barneveld, R. J., Carter, C. J., Tivey, D. R. and Brooker, J. D. (1999). Development of an invitro assay for the assessment of alternative protein sources for use in artificial diets for farmed Southern Bluefin Tuna (*Thunnus maccoyii*). Fisheries Research and Development Corporation Final Report, Project 95/068.

van Barneveld, R. J., Buchanan, J. and Ellis, D. (2009). Optimisation of farmed southern bluefin tuna (*Thunnus maccoyii*) nutrition to improve feed conversion efficiency and reduce production costs. Aquafin CRC, Adelaide, pp. 1-198.

Vandenberg, G. W. and De La Noüe, J. (2001). Apparent digestibility comparison in rainbow trout (*Oncorhynchus mykiss*) assessed using three methods of faeces collection and three digestibility markers. *Aquaculture Nutrition.* **7**, 237-245.

Vega, R., Valdebenito, I., Alfaro, D. and Cardenas, S. (1994). Effect of feeding frequency on growth and survival of rainbow trout (*Oncorhynchus mykiss*) farmed at Lautaro Fish Farming Experimental Station, Chile. *Aquaculture.* **124**, 291.

Vera, L. M., De Pedro, N., Gómez-Milán, E., Delgado, M. J., Sánchez-Muros, M. J., Madrid, J. A. and Sánchez-Vázquez, F. J. (2007). Feeding entrainment of locomotor activity rhythms, digestive enzymes and neuroendocrine factors in goldfish. *Physiology and Behaviour.* **90**, 518-524.

Videl, A.T., De la Gándara García, F., García Gómez, A. and Jover Cerdá, M. (2008). Effect of the protein/energy ratio on the growth of Mediterranean yellowtail (*Seriola dumerili*). *Aquaculture Research.* **39**, 41-48.

Wang, N., Xu, X and Kestemont, P. (2009). Effect of temperature and feeding frequency on growth performances, feed efficiency and body composition of pikeperch juveniles (*Sander lucioperca*). *Aquaculture*. **289**, 70-73.

Ward, D. A., Carter, C. G., and Townsend, A. T. (2005). The use of yttrium oxide and the effect of faecal collection timing for determining the apparent digestibility of minerals and trace elements in Atlantic salmon (*Salmo salar,* L.) feeds. *Aquaculture Nutrition.* **11**, 49-59.

Wardle, C. S., Videler, J. J., Arimoto, T., Franco, J. M. and He, P. (1989). The muscle twitch and the maximum swimming speed of giant bluefin tuna, *Thunnus thynnus* L. *Journal of Fish Biology.* **35**, 129-137.

Watanabe, K., Aoki, H., Yamagata, Y., Kiron, V., Satoh, S. and Watanabe, T. (2000). Energy and protein requirements of yellowtail during winter season. *Fisheries Science.* **66**, 521-527.

Williams, I., Williams, K. C., Smith, D. M. and Jones, M. (2006). Polka-dot grouper, *Cromileptes altivelis,* can utilize dietary fat efficiently. *Aquaculture Nutrition.* **12**, 379-387.

Woods, S. C. and Seeley, R .J. (2000). Adiposity signals and the control of energy homeostasis. *Nutrition*. **16**, 894–902.

Yamamoto, T., Shima, T., Furuita, H. and Suzuki, N. (2002). Influence of feeding diets with and without fish meal by hand and by self-feeders on feed intake, growth and nutrient utilization of juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquaculture.* **214**, 289-305.

Young, A., Morris, P. C., Huntingford, F. A. and Sinnott, R. (2005). The effects of diet, feeding regime and catch-up growth on flesh quality attributes of large (1+ sea winter) Atlantic salmon *Salmo salar*. *Aquaculture.* **248**, 59-73.

Young, J. W., Bradford, R. W., Lamb, T. D. and Lyne, V. D. (1996). Biomass of zooplankton and micronekton in the southern bluefin tuna fishing grounds off eastern Tasmania, Australia. *Mar Ecol Prog Ser.* **138**, 1-14.

Young, J. W., Lamb, T. D., Le, D., Bradford, R. W. and Whitelaw, A. W. (1997). Feeding ecology and interannual variations in diet of southern bluefin tuna, *Thunnus maccoyii*, in relation to coastal and oceanic waters off eastern Tasmania, Australia. *Environmental Biology of Fishes.* **50**, 275-296.